Influence of Genetic Polymorphisms on Cognition, Sleep, and Sleep Homeostasis in Healthy Adults

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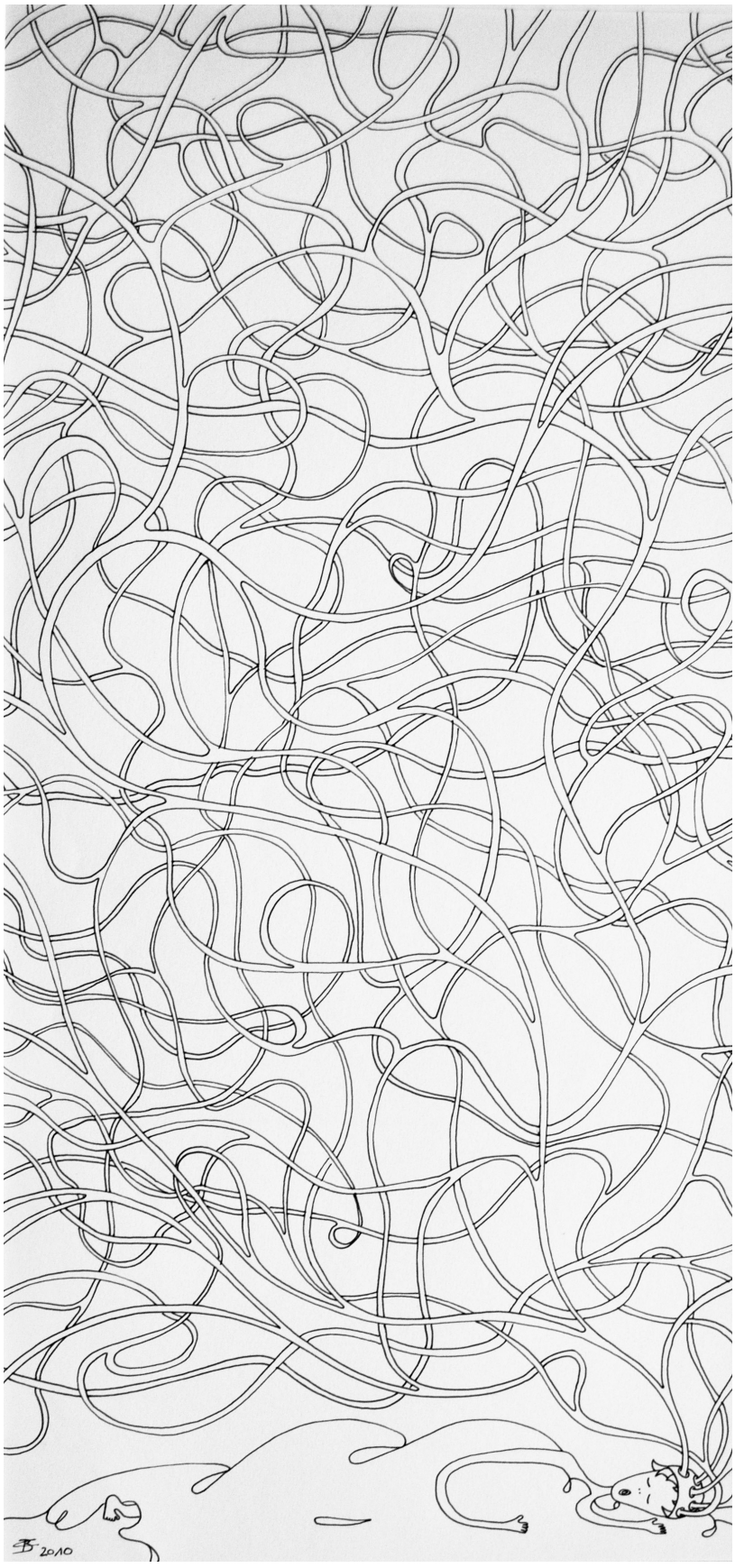
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"The human brain is the most fascinating
three pounds of matter on this planet,
maybe even in the universe."

Pam Schiller

Preface

The scientific research reported in the current thesis was performed in the Section of Chronobiology and Sleep Research at the Institute of Pharmacology and Toxicology of the University of Zurich. Most of all, I am deeply grateful to Prof. Dr. Hans-Peter Landolt, the principal investigator for giving me the opportunity to perform my PhD in his group. In addition, I would like to warmly thank Prof. Dr. Peter Brugger for his individual support during the project. Both introduced me carefully and with a lot of patience to the field of basic research, guided me through the different steps of my projects and advised me in the preparation of scientific talks, posters and manuscripts. I thank Prof. Dr. Jean-Marc Fritschy for being the examiner of my thesis, Prof. Dr. Thierry Hennet and Prof. Dr. Christoph Michel for being my co-examiners. I would further thank the members of the human sleep group, who supported me in many aspects during my thesis. Especially Sereina, who introduced me very carefully and closely to the different methods used in sleep research and genotyping and who has always been a good friend. A warm thank goes to Kathrin, Alessia, Sabine, Federica, Renate, Thomas, Carina, Sarah and Sebastian, who supported me during the sleep studies and data analyses and who were also good company during coffee times. Thanks go to Prof Dr. Achermann, Dr. Roland Dürr and Karl Wütrich for technical support and helping with data analyses. Thanks to Prof. Dr. Steven Brown and his group for giving me the opportunity to conduct the DNA extractions in their laboratory. Thanks to Susanne Huber from the Clinical Psychology and Psychotherapy Lab of the University of Zurich for helping me with the analysis of α-amylase activity. Moreover, I appreciated the pleasant collaboration with Prof. Dr. Wolfgang Berger at the Institute of Medical Genetics of the University of Zurich. Most notably, I would like to thank Dr. Nikolaus Schäfer for his help with genotyping. I further like to thank Jurian, Barbara, Lucas, Istvan, and Fabian for their kind assistance when necessary and for the pleasant atmosphere in the lab. Special thanks to Peeyusha, Friedel and Mustafa, who supported me with cognitive testing of the 243 subjects. Thanks to Sigal, Isabelle, Alice, Vero and Lea who supported me during the collection and analyses of Actimetry data. A special thank goes to Dr. Leila Tarokh for helpful comments on the manuscript and to the ZIHP for the financial support.

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Summary

Cognitive abilities, habitual sleep and sleep-wake regulation show trait-like individual differences among healthy people. Whether the share a common neurobiological basis was one of the principal aims of the present dissertation. In addition, distinct genetic polymorphisms contributing to inter-individual differences in brain plasticity and sleep-wake mechanisms were studied.

To this end, performance on cognitive tasks was examined in 243 healthy adults and subjective sleep duration was estimated using the Munich Chronotype Questionnaire. The large data set with various measures from different cognitive domains was used to investigate in the first part of the project whether functional hemispheric asymmetries of the prefrontal cortex were associated with the extent of a well-known bias in the production of numbers "at random" (chapter 2). Analysis of the random number generation (RNG) task confirmed the presence of this small number bias (SNB) in 209 right-handers (102 women, 107 men). Crucially, these subjects, who showed an above average advantage for design fluency (right-hemisphere mediated) over letter fluency (left-hemisphere mediated), exhibited a significantly more pronounced SNB. This association indicates that asymmetric prefrontal cortex functions contribute to asymmetries in a highly abstract number space in healthy subjects. Next, we examined with the same data set, whether prefrontal executive functioning and habitual sleep duration are mutually related (chapter 3). In addition rest-activity patterns during 4-week wrist-actigraphy were collected in 35 women and 47 men. Based on median-split analysis, we found that better performance on a Stroop Color-Word task was associated with shorter habitual sleep duration during leisure days. The task probes the executive functions interference/inhibition control. Furthermore, attention variability (d2 attention task) and response stereotypy (adjacency on the RNG task) were related to self-reported, but not to wrist-actigraphy-derived sleep duration during leisure days. Measures of learning and memory were not associated with habitual sleep duration. Notably, comparison of self-reported and actigraphic measures revealed that individuals overestimated habitual sleep length during leisure days. The third and fourth part of the dissertation addressed the potential roles for functional single nucleotide polymorphisms on neurobehavioral performance and sleep-wake regulatory mechanisms in healthy humans. First, we studied the impact of the functional p.Val66Met polymorphism of brain-derived

neurotrophic factor (*BDNF*) on waking and sleep-wake regulatory mechanisms (chapter 4). Animal data suggest that BDNF is causally related to sleep homeostasis. To address this question, 22 subjects were studied according to a matched-pair design. The protocol consisted of a baseline night, 40 hours of prolonged wakefulness, and a recovery night. Consistent with previous findings, individuals with the variant Met allele (Val/Met) performed worse on a 2-back task working memory task than Val/Val allele carriers. Subjective sleepiness was similar in both groups. Deep stage 4 sleep and EEG slow-wave activity (SWA: 0.75 - 4.5 Hz) in most bipolar EEG derivations along the antero-posterior axis in nonREM sleep were reduced in Val/Met compared to Val/Val genotype in both nights. In addition, the build-up of SWA in nonREM sleep and alpha activity in wakefulness (10 - 11.5 Hz) were reduced in the former than the latter. In summary, the *BDNF* genotype predicts robust frequency- and vigilance-state specific effect on the EEG in waking and sleep. Nevertheless, the dynamics of the homeostatic process was similar in both groups. Next, we examined in a similar approach, whether the p.Asp8Asn polymorphism of the enzyme adenosine deaminase (*ADA*) affects waking performance and interferes with sleep homeostasis (chapter 5). Previous data demonstrate that the adenosinergic system is important in sleep-wake regulatory mechanisms. Analyses revealed that in rested and sleep-deprived state, carriers of the variant Asn allele (11 Asp/Asn genotype) performed worse than carriers of two Asp alleles (11 Asp/Asp genotype) on tasks of attention (d2 attention task and psychomotor vigilance task (PVT). In addition, fatigue was higher and vigour was lower in Asp/Asn genotype than in Asp/Asp genotype. Slow wave sleep, low-frequency delta (0.75 - 1.5 Hz) activity in nonREM sleep, and alpha activity (8.5 – 12 Hz) in wakefulness were higher in both nights in the former when compared to the latter. We conclude that a distinct variation in an enzyme affecting adenosine metabolism is an important determinant of waking and sleep quality. In contrast, sleep homeostasis and habitual sleep duration were not affected by the *ADA* genotype. In summary, these findings provide new insights to the possible neurochemical mechanisms influencing cognitive performance and sleep-wake regulation. Notably, it has been shown that the frontal cortex not only contributes to inter-individual differences in executive functioning, including numerical cognition, but also to sleep regulatory mechanisms. Our data support a role for sleep in waking functions, in particular in plastic synaptic processes and that genetic polymorphisms play an important role in trait-like inter-subject variation in both, cognitive and sleep-wake regulatory processes.

Zusammenfassung

Die vorliegende Dissertation untersuchte, ob kognitive Fähigkeiten und die Schlaf-Wachregulation eine gemeinsame neurobiologische Grundlage haben. Vertiefend waren wir daran interessiert, ob bestimmte funktionelle Polymorphismen zu inter-individuellen Unterschieden bezüglich kognitiver Fähigkeiten und der Schlaf- Wachhomöostase beitragen.

Zu diesem Zweck wurden die kognitiven Fähigkeiten von 243 gesunden Erwachsenen getestet und die habituelle Schlafdauer mittels des Munich Chronotype Fragebogens (MCTQ) erfragt. Aufbauend auf diesem Datensatz haben wir in einer ersten Studie untersucht, ob funktionelle, hemisphärische Asymmetrien des präfrontalen Kortex mit der Präferenz für kleine Zahlen zusammenhängen (Kapitel 2). Dazu haben die Teilnehmer im Randum Number Generation (RNG) Test zufällig Zahlen von 1 bis 6 generiert, ähnlich dem Würfeln. Des weitern zeigten besonders Individuen mit überdurchschnittlich gutem Abschneiden in figuralen Aufgaben (rechte Hemisphäre) eine Vorliebe für kleine Zahlen – dies im Gegensatz zu jenen mit besseren Leistungen in verbalen Aufgaben (linke Hemisphäre). Zudem konnte die generelle Vorliebe, mehr kleine Zahlen als grosse Zahlen zu generieren, konnte auch bei 209 Rechtshändern (102 Frauen, 107 Männer) bestätigt werden. Dieser Zusammenhang lässt vermuten, dass bei gesunden Personen asymmetrische Funktionen im präfrontalen Kortex zu Asymmetrien im abstrakten Zahlenraum beitragen.

In der zweiten Studie wurde der Zusammenhang zwischen präfrontalen Exekutivfunktionen und der habituellen Schlafdauer untersucht (Kapitel 3). Neben den bereits erhobenen Daten, wurde das Ruhe-Aktivitätsmuster von 35 Frauen und 47 Männern während vier Wochen mit Hilfe eines Aktivitätsmonitors ermittelt. Mittels Median-split Analyse konnten wir zeigen, dass bessere Inhibitions/Interfenzkontrolle im Stroop Color-Word Test mit einer kürzeren habituellen Schlafdauer während arbeitsfreien Tagen zusammenhängt. Darüber hinaus waren Aufmerksamkeitsvarianz (d2 Aufmerksamkeitstest) und Stereotypie (ein Messparameter des RNG Tests) mit einer kürzeren Schlafdauer assoziiert, wobei dieser Zusammenhang nur für die subjektive und nicht für die objektive Schlafdauer galt. Im Gegensatz dazu waren Lern- und Gedächtnisleistungen nicht von der angegebenen habituellen Schlafdauer abhängig. Der Vergleich der subjektiven (Fragebogen) mit der

objektiven Schlafdauer zeigte, dass Männer wie Frauen ihre habituelle Schlafdauer am Wochenende generell überschätzen.

Im dritten und viertel Teil der Dissertation wurde der Einfluss funktioneller Polymorphismen auf die kognitive Leistungsfähigkeit und das Schlaf-Wachverhalten untersucht. Das Studienprotokoll bestand aus zwei Kontrollnächten, gefolgt von einem 40-stündigen Schlafentzug und einer Erholungsnacht. Zuerst wurde der Einfluss des funktionellen brain-derived neurotrophic factor (BDNF) p.Val66Met Polymorphismus auf die Auswirkung von verlängerter Wachzeit auf das Arbeitsgedächtnis, auf die subjektive Befindlichkeit und auf das Schlaf- und Wach-Elektroenzephalogramm (EEG) überprüft. In Tierstudien wurde bereits ein kausaler Zusammenhang zwischen BDNF und der Schlaf-Homöostase gezeigt, nicht aber im Mensch. Um diesen Zusammenhang im Menschen zu untersuchen, wurden 11 Personen mit der Genvariante Met (Val/Met) mit 11 Val/Val Alleleträgern gepaart und im Schlaflabor untersucht. Die Befunde bestätigten, dass Val/Met Individuen ein schlechteres Arbeitsgedächtnis im 2-zurück Tests aufweisen als Val/Val Individuen. Die subjektive Befindlichkeit unter Schlafentzug war bei beiden Gruppen gleich. Tiefschlaf Stadium 4 und die langsamwellige Deltaaktivität (0.5 – 4.5 Hz) im nonREM Schlaf, ein Mass für die Schlafintensität waren während der Kontrollnacht wie auch während der Erholungsnacht in den EEG Ableitungen entlang der antero-posterioren Achse bei den Val/Met Individuen im Vergleich zu den Val/Val Individuen reduziert. Die Alphaaktivität (10 – 11.5 Hz) im Wach-EEG war in den Met Alleleträgern ebenfalls geringer. Aufgrund dieser Resultate kommen wir zum Schluss, dass der BDNF p.Val66Met Polymorphismus bei gesunden Erwachsenen nicht nur einen Einfluss auf das Arbeitsgedächtnis hat, sondern auch zur physiologischen Regulation des Schlafs beiträgt.

Als letztes haben wir in einem sehr ähnlichen Protokoll untersucht, ob der funktionelle p.Asp8Asn Polymorphismus des Enzyms Adenosin Deaminase (ADA) einen Einfluss auf Aufmerksamkeitsleistungen und die homöostatische Schlaf- Wachregulation haben (Kapitel 5). Diverse Studien haben gezeigt, dass adenosinerge Mechanismen zu inter-individuellen Unterschieden in der Schlaf-Wachregulation beitragen. Untersuchungen bei 10 Frauen und 12 Männer haben gezeigt, dass Asp/Asn Individuen im Vergleich zu homozygoten Asp/Asp Individuen, eine stark eingeschränkte Aufmerksamkeit/Vigilanz (d2 Aufmerksamkeitstest und psychomotorischer Vigilanztest (PVT)) und eine erhöhte subjektive Schläfrigkeit unter normalen Bedingungen wie auch unter Schlafentzug aufweisen. Die langsamwelligen

Deltaaktivität (0.75 – 1.5 Hz) im nonREM Schlaf und die Alphaaktivität (8.5 – 12 Hz) im Wach-EEG waren bei den Asp/Asn Individuen sowohl in der Baseline als auch in der Erholungsnacht erhöht. Diese Befunde weisen darauf hin, dass eine reduzierte Enzymaktivität im adenosinergen System zu Veränderungen in der Aufmerksamkeit führt und die Schlafintensität beeinflusst. Entgegen dessen bleiben die Dynamik der Schlaf-Wachregulierung und die habituelle Schlafdauer unbeeinflusst.

Zusammengefasst liefert meine Doktorarbeit Hinweise auf spezifische neurochemische Mechanismen, welche in der kognitiven wie auch in der Schlaf-Wachregulation eine wichtige Rolle spielen. Zudem konnten wir zeigen, dass der präfrontale Kortex nicht nur für inter-individuelle Unterschiede in Exekutivfunktionen, einschliesslich numerischer Kognition, zuständig ist, sondern auch eine Rolle in der Schlaf- Wachregulation spielt. Schlussendlich konnte dargelegt werden, dass funktionelle Polymorphismen einen starken Einfluss auf die individuell unterschiedlichen Eigenschaften kognitiver Fähigkeiten und die Schlaf-Wachregulation haben.

Abbreviations

5-HT	Serotonin
5'-EN	5'-extonucleotidase
5'-N	5'-nucleotidase
AAS	Ascending activating system
Ach	Acetylcholin
ADA	Adenosine deaminase
ADORA2A	Adenosine A_{2A} receptor gene
ADP	Adenosine-diphosphate
AK	Adenosine kinase
AMP	Adenosine-monophosphate
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
Asn	Asparagine
Asp	Aspartic acid
AT	Nucleoside transporters of adenosine
ATP	Adenosine-triphosphate
BDNF	Brain-derived neurotrophic factor
BF	Basal forebrain
Bf-S	Befindlichkeits-Skala
CI	Confidence interval
COMT	Catechol-o-methyltransferase
CP	Centroparietal EEG derivation
Cx	Cortex
ECG	Electorcardiogram
EEG	Electroencephalography
EMG	Electromyogram
EOG	Electrooculogram
ER	Endplasmic reticulum
ESS	Epworth Sleepiness Scale
FC	Frontocentral EEG derivation
FFT	Fast Fourier transformation
fMRI	Functional magnetic resonance imaging
GABA	γ-amino-butyric-acid

Hcrt	Hypocretin
His	Histamine
IQ	Intelligence quotient
LC	Locus coeruleus
LDT	Laterodorsal tegmentum
LTP	Long-term potentiation
mBDNF	Mature brain derived-neurotrophic factor
MCTQ	Munich ChronotypeQuestionnaire
Met	Methionine
NA	Noradrenaline
nonREM	Non-rapid eye movement
ORX	Orexin
p75NTR	Pan neurotrophin receptor
PCR	Polymerase chain reaction
PET	Positron emission tomography
PO	Parieto-occipital EEG derivation
POMS	Profile of mood states
PPT	Pedunculopontine tegmentum
PVT	Psychomotor vigilance task
QTL	Quantitative trait loci
RAVLT	Rey Auditory Verbal Learning Test
REM	Rapid eye movement
RF	Reticular formation
RL	REM latency
RNG	Random number generation
RVDLT	Rey Visual Design Learning Test
SD	Sleep deprivation
SEFF	Sleep efficiency
SL	Sleep latency
SLEP	Sleep episode
SNB	Small number bias
SNP	Single nucleotide polymorphism
SNARC	Spatial Numerical Association of Response Codes
SSS	Stanford Sleepiness Scale
SWA	Slow-wave activity

SWS	Slow wave sleep
ThCx	Thalamocortical neurons
TMN	Tuberomammillary nucleus
TMS	Transcranial magnetic stimulation
TrkB receptor	Neurotrophic tyrosine kinase receptor B (type 2)
TST	Total sleep time
Val	Valine
VLPO	Ventrolateral preoptic area
VNTR	Variable number tandem repeat
vPAG	Ventral periaqueductal gray matter
WASO	Wakefulness after sleep onset

Chapter 1

Introduction

1.1. Functional organisation of the human cortex

1.1.1. The cerebral cortex – a rough overview of its functional organization

The cerebrum or cortex forms the largest division of the human brain and is associated with higher brain functions such as planning and execution of actions. It is anatomically divided into two hemispheres, each of which is grossly divided into four lobes (Fig. 1).

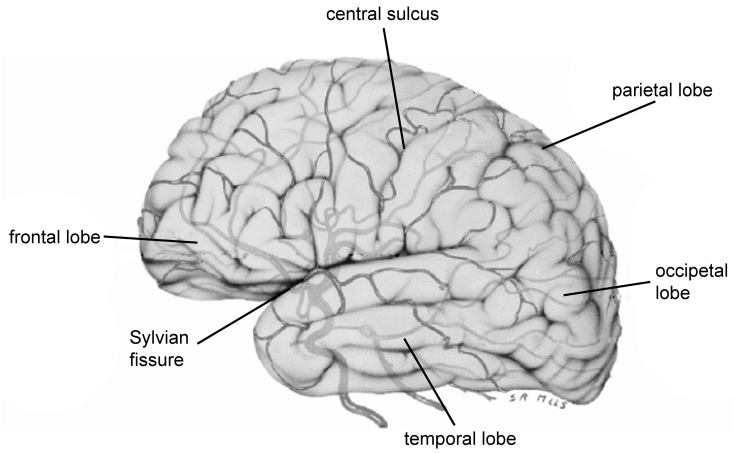

Figure 1: The human cerebral cortex is divided into four lobes (adapted from Roche Lexikon Medizin 5.0)
The occipital lobes, located at the back of the brain are concerned with many aspects of vision. The parietal lobes, located behind the central sulcus are associated with somatosensory perception. The temporal lobes located below the sylvian fissure (=lateral sulcus) obtain auditory and memory functions. The frontal lobes, located in front of the central sulcus are associated with reasoning, planning, parts of speech and motor execution, emotions, and problem solving.

Each lobe includes many different functions. The occipital lobes are associated with visual perception and processing. The parietal lobes play an important role in orientation, spatial awareness and somatosensory perception. The temporal lobes are important for auditory, visual and memory functions (hippocampus). The frontal lobes are associated with memory formation, motor execution and executive functioning. More specifically, executive functions such as planning, reasoning, personality, decision making, interference control and moderating correct social behavior rely on the prefrontal cortex, which is localized in the

anterior part of the frontal lobes, anterior to the motor and premotor areas (Kandel et al., 2000, Gazzaniga et al., 2002). With respect to sleep, it was proposed that the frontal regions of the brain are especially sensitive to sleep deprivation (Horne, 1993). For example, healthy individuals suffering from sleep deprivation exhibit cognitive deficits, which are similar to those observed in patients with prefrontal cortical lesions. After insufficient sleep they show increased perseveration, impaired planning abilities or flatness of speech (Harrison and Horne, 1996, 1997, Harrison et al., 2000).

1.1.2. Functional asymmetries of the cerebral hemispheres

Hemispheric asymmetries are a widespread phenomenon among various species (Gunturkun, 1997, Hellige and Marks, 2001, Rogers and Andrew, 2002). Research suggests that human cortical asymmetries are caused by asymmetric gene transcription in the embryonic left and right cerebral cortices (Sun et al., 2005). However, while cytoarchitectonically the similarities clearly outweigh any differences, functional hemispheric differences are so marked that they are readily observable to laypeople (e.g. the asymmetry underlying a person's handedness). Furthermore, the two hemispheres are specialized for distinct perceptual and cognitive functions. Most well known is the specialization of the right hemisphere for spatial and facial recognition and the left hemisphere for tool use ("praxia") and language abilities (Kelley et al., 1998, Golby et al., 2001, Powell et al., 2005). Patients with spatial neglect following right hemisphere damage exhibit deficits in the perception of forms and objects only to the left of their body. Although much progress has been made, the exact nature of spatial neglect is still poorly understood. Healthy people, interestingly exhibit right-cerebrally dominance for some nonverbal functions, such as spatial attention and the processing of faces (Uttl and Pilkenton-Taylor, 2001, Bottini and Toraldo, 2003). This phenomenon of right-hemisphere dominance in the control of spatial attention is also evident in birds, picking food first from the left and later from the right side of hemispace (Diekamp et al., 2005). This bias towards the left hemispace is often referred to as "pseudoneglect" (Oliveri et al., 2004, Gobel et al., 2006), and is explained by hyper-attention towards the left visual field or hemispace. Recently, a study in healthy adults provided evidence that genetic variation in dopaminergic systems modulates lateral biases in spatial attention (Greene et al., 2010), indicating that

pseudoneglect might be at least in part genetically determined. A more general discussion about different genetic background in terms of cognition can be found in chapter 1.6.
It is suggested that pseudoneglect is also relevant in number space. If subjects are required to generate random sequences of numbers they preferentially select more smaller then larger numbers (Loetscher and Brugger, 2007). This observation may indicate a spatial component in random number generation (RNG). Further evidence for a spatial element in RNG was provided by an experiment, showing that RNG is systematically influenced by lateral head turn (Loetscher et al., 2008). If subjects turned their head to the left side of space they produced more smaller than larger numbers. In addition, it is widely accepted that number concepts are represented bilaterally in the parietal lobes (Hubbard et al., 2005). In contrast, frontal cortical contribution to numerical cognition has been somewhat overlooked, although studies on RNG tasks in humans (Daniels et al., 2003, Loetscher and Brugger, 2007) and in non-human primates (Nieder, 2009, Bongard and Nieder, 2010) strongly support the notion that the contribution of the frontal cortex is important as well. Asymmetric prefrontal cortex functions in relation to spatial-numerical interaction will be discussed in detail in chapter 2.

1.2. Common neurobiological basis of cognition and sleep ?

Cognitive abilities and sleep variables show trait-like individual differences among healthy people (Egan et al., 2003, Van Dongen et al., 2005, Buckelmüller et al., 2006, Landolt, 2008a). It is known that insufficient sleep leads to increased sleepiness and impairments of cognitive and metabolic functions. Networks of executive functions, mainly localized in the prefrontal cortex, appear to be particularly vulnerable to sleep deprivation, suggesting that sleep deprivation primarily affects the frontal lobes (Dorrian et al., 2005, Goel et al., 2009). For example, a study of the effects of sleep deprivation on a RNG task, which relies heavily on the prefrontal cortex, revealed that prolonged wakefulness is associated with a decrease in the number of responses and an increase in response stereotypy (Gottselig et al., 2006). In addition, impairments of attention due to increased daytime sleepiness results in increased lapses of attention in both frequency and duration, as measured by the psychomotor vigilance task (PVT) (Dinges et al., 1997, Adam et al., 2006, Lim and Dinges, 2008). The PVT is a reliable and well-established task to measure the consequences of sleep loss in brain networks associated with attentional functions (Drummond et al., 2005). Not

only attentional networks, but also working memory and frontal executive function networks are affected by sleep deprivation (Goel et al., 2009). Only minor disruptions of sleep that diminish slow wave sleep (SWS) are sufficient to impair memory encoding and reduce hippocampal activation (Van Der Werf et al., 2009).

Concerning electroencephalography (EEG), prolonged waking in adults increases EEG power in subsequent recovery sleep in the low frequency ranges (1 – 10.75 Hz) with the largest increase in the frontal region of the cortex and a decrease in power in the sigma band (13 – 15.75 Hz) (Finelli et al., 2001b). Interestingly, neuroimaging studies using functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) claim that sleep loss modifies activation in distinct brain areas (Goel et al., 2009): It either decreases activation in distinct brain areas, as for example the prefrontal cortex, along with impaired cognitive performance (Drummond et al., 1999, Thomas et al., 2000) or increases activation in the prefrontal cortex and parietal lobe relative to well-rested state, along with relatively unaffected or even better cognitive performance (Drummond et al., 2000, Chee and Choo, 2004).

Intriguingly, negative mood states, such as increased fatigue, loss of vigor, confusion or sleepiness after sleep deprivation appear to be stable and reliable within subjects (Goel et al., 2009). In contrast, there exists high inter-individual variability in the degree of vulnerability to sleep deprivation (Van Dongen et al., 2004, Goel et al., 2009). Part of this differential vulnerability may be trait-like and reflects genetic influences (Landolt, 2008a, Tafti, 2009), which will be discussed in chapter 1.6. In general, the precise role of sleep in memory and brain plasticity is poorly understood. However, sleep deprivation studies are a powerful approach to gain insights in sleep-wake regulatory mechanisms and brain plasticity.

Nevertheless, behavioural and electroencephalographic measures have shown that sleep in adults plays an important role in memory consolidation and learning (Maquet, 2001, Walker and Stickgold, 2006). The importance of sleep in cognitive development and learning is already seen in early childhood (Roffwarg et al., 1966). Children exhibit a high seep quotient, which is suggested to indicate that sleep actively supports cognitive development. The assumption that sleep is important for brain plasticity was recently shown in 1-to 2-day old sleeping newborns. Their learning abilities were tested by repeated tones that were followed by a gentle puff of air to their eyelids. After about 20 minutes, 24 of the 26 babies

squeezed their eyelids together when the tone was sounded without the puff of air, reflecting a learning effect (Fifer et al., 2010).

1.2.1. Synaptic homeostasis hypothesis

A close relationship between sleep, local brain plasticity and cognitive processes is proposed by the synaptic homeostasis hypothesis of sleep (Tononi and Cirelli, 2003, 2006) (Fig. 2).

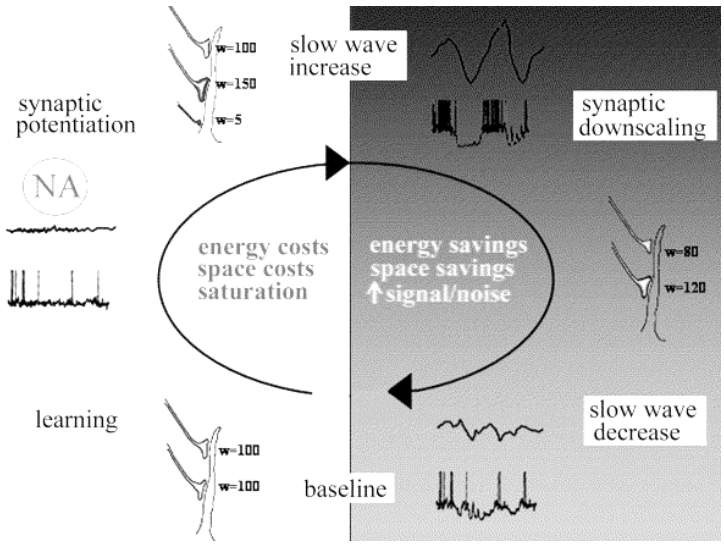

Figure 2: Synaptic homeostasis hypothesis (adapted from Tononi and Cirelli, 2006)
The hypothesis posits that during wakefulness (white background) synaptic strength increases in many circuits of the brain as a result of learning and adaptation to the environment. The EEG is activated and the neuromodulatory milieu (e.g. high levels of noradrenaline (NA)) favors synaptic potentiation. This is associated with high energy and space requirements and neuronal saturation. Across a night of sleep (grey background), while being disconnected from the environment, changes in the neuromodulatory milieu trigger the occurrence of slow oscillations in membrane potential, which are reflected in the EEG as slow-wave activity (SWA: 0.75 – 4.5 Hz). Because average synaptic strength at the end of the waking period is high, the initial SWA shows high amplitude slow-waves. During the course of the night SWA declines in amplitude, which reflects synaptic downscaling. Thus, the hypothesis suggests that the function of sleep is to set synaptic strength to an appropriate baseline level and to get the synapses leaner and more efficient after a day of wakefulness to preserve information, which is reflected in an increase of the signal-to-noise ratio. Therefore, sleep is important to maintain a balance of synaptic strength and to allow acquisition of information throughout life.

According to this hypothesis, the homeostatic regulation of sleep as measured by slow-wave activity (SWA; EEG power within 0.5 - 4.5 Hz), a proposed EEG correlate of sleep intensity (Borbely, 1982) is directly related to the amount of synaptic potentiation, which occurs during wakefulness (Tononi and Cirelli, 2003, 2006). The hypothesis predicts that the strength of each cortico-cortical synapse, potentiated during the day while awake, is decreased ("downscaled") by a certain factor during sleep. According to this hypothesis

synaptic downscaling would be tied to the dynamics of SWA as an electrophysiological marker of synaptic strength. This process would be responsible for keeping the overall synaptic weight in balance and may represent a key mechanism for the modulation of cognitive functioning (Turrigiano and Nelson, 2000).

Long-term potentiation (LTP) of synapses is now considered the major candidate mechanism underlying learning and cognitive functions (Bliss and Lomo, 1973, Borroni et al., 2000, Lu et al., 2008, Minichiello, 2009). Several studies in animals investigating the expression of plasticity-related genes, such as Arc, the brain-derived neurotrophin (*BDNF*) or the phosphorylated CRE-binding protein (P-CREB) support the hypothesis that expression is upregulated in the brain of waking animals and downregulated during sleep (Cirelli and Tononi, 2000, Huber et al., 2007, Hanlon et al., 2009). Additionally, it is presumed that the increase of plasticity related gene expression depends on the duration of wakefulness (reviewed in Cirelli et al. (2002)). Moreover, it is hypothesized that SWA homeostasis reflects synaptic changes underlying learning and that these changes induce higher SWA during subsequent sleep in the brain areas involved in learning (Huber et al., 2004, 2006). For example, when healthy subjects completed a rotation adaptation task shortly before going to sleep, a local increase in SWA over the areas in the cortex responsible for task performance was found during subsequent sleep, when compared to a control task, where no rotation was administered (Huber et al., 2004). Furthermore, the amount of local increase in SWA was positively related to the enhancement of performance after sleep. In contrast, immobilizing a subject's arm during wakefulness decreased somatosensory and motor evoked potentials, which reflects local synaptic depression (Huber et al., 2006). This decrease was consistent with the reduction of SWA in the contralateral sensorimotor cortex. Taken together, these studies suggest that slow-waves in non-rapid eye movement (nonREM) sleep are closely associated with synaptic homeostasis and that local differences in homeostatic sleep-wake regulation reflect the previous waking history in a use-dependent manner. Whether improvement of performance is causally related to specific sleep parameters remains to be further investigated (Landsness et al., 2009).

Interestingly, it was recently suggested that habitual sleep duration in adolescents is negatively associated to IQ scores (Geiger et al., 2010). According to the synaptic homeostasis hypothesis, we hypothesize that better tolerance of high sleep pressure, as exhibited in short sleepers (Aeschbach et al., 2000, Aeschbach et al., 2001) would permit an

increased time for the acquisition of cognitive processes. Whether executive functions may be associated to habitual sleep duration in healthy middle-aged adults will be discussed in detail in chapter 3.

1.3. Homeostatic and circadian regulation of sleep

The question why we sleep is still not answered. This question has been of interest to sleep scientists for centuries and yet there is still no satisfying answer. Sleep is an actively regulated process and not, as it was long believed, a passive state solely defined by the absence of wakefulness and reduced brain activity.

Two main processes are postulated to be involved in sleep-wake regulation, the circadian pacemaker process C and the homeostatic process S (Borbely, 1982) (Fig. 3). Process C is driven by a clock-like mechanism located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Moore and Eichler, 1972, Stephan and Zucker, 1972). This master clock is basically independent of prior sleep and waking and oscillates with an endogenous period of roughly 24 hours. It is responsible for the temporal organization of sleep and wake (Dijk and Czeisler, 1994). The second process, the sleep-dependent homeostatic process S, reflecting the prior sleep-wake history, increases according to an exponential saturating function during wakefulness and declines roughly exponentially during sleep (Achermann, 2004). The interaction of these two processes is proposed to generate the timing of sleep and waking (Borbely, 1982) and to maintain wakefulness and alertness during the day and to consolidate sleep throughout the night (Van Dongen and Dinges, 2003).

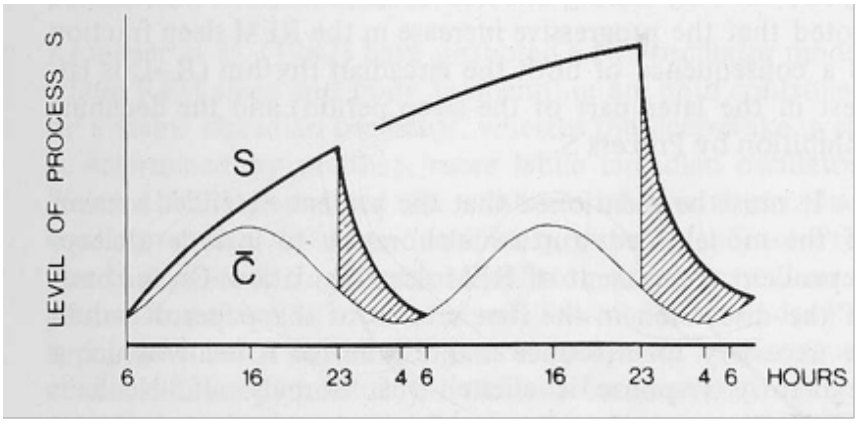

Figure 3: The two-process model (from Borbély et al., 1982)
Schematic view of the two-process model of sleep-wake regulation during regular and extended wakefulness. As a function of time, the time courses of process S and the negative function of process C (curve C) are plotted. The interaction of the two processes is proposed to generate the timing of sleep and waking.

Physiological markers reflecting sleep homeostasis are sleep duration and sleep intensity. Sleep intensity is thought to be reflected by SWA in nonREM sleep. SWA increases as a function of prior wakefulness mediating the rise in "sleep pressure" and decreases in nonREM sleep episodes (Borbély and Achermann, 2005). The increase of sleep propensity during wakefulness was associated to the rise of theta/low alpha activity in the waking EEG, which reflects integrated EEG power density values of frequencies between 5 and 9 Hz (Cajochen et al., 1995, 2001, Finelli et al., 2001b).

1.4. Analysis of the electroencephalogram in sleep and wakefulness

The German psychiatrist Hans Berger (1929) was the inventor of the EEG. He was the first to record electroencephalograms from humans and is the discoverer of the rhythmic alpha activity.

The electrical activity of the brain induces alternating electrical potential differences at the scalp surface, which can be measured by the EEG. Potential differences can be calculated between an active and a reference electrode or between two active electrodes, respectively (Kandel et al., 2000). In conjunction with simultaneous measurements of eye movements (electrooculogram; EOG) and chin muscle tone (electromyogram; EMG), it is possible to discriminate between three major vigilance states: wakefulness, non-rapid eye movement (REM) sleep and REM sleep. A systematic method allowing for the description of sleep structure was developed by Rechtschaffen and Kales (1968). They proposed a standardised terminology to allow visual discrimination between the three major vigilance states (wakefulness, nonREM sleep and REM sleep). The scoring rules are mainly based on the frequency, amplitude and morphology of the EEG waves. However, the conventional method of visual scoring is not sufficient to quantify brain physiology because of the rather general and arbitrary criteria used. Thus, to obtain quantitative information about the physiological mechanisms that underlie the changes in the EEG, spectral analysis is used. This powerful method calculates the power spectra of the EEG by Fast-Fourier-Transformation (FFT; Cooley and Tukey (1965)). The algorithm transforms and integrates a biological, digitised EEG signal from the time domain into the frequency domain by decomposing it into sine and cosine functions of varying amplitude and frequency per time window. This kind of analysis requires a stationary signal. Therefore, short time windows are defined to obtain a quasi-stationary signal, because EEG waves are not stationary. For the

analysis of the sleep EEG 4-s windows are subjected to spectral analysis and for the analysis of the waking EEG 2-s windows. Subsequent FFT of these short time segments results in a 0.25 Hz frequency resolution of the sleep power spectrum, and in a 0.5 Hz frequency resolution of the waking power spectrum. The resulting power spectrum depicts power as a function of frequency bin (e.g. μV^2/Hz) and reflects the distribution of EEG power over a certain time (e.g. across a night). As a consequence dominant rhythmic activity in the EEG is reflected as peaks in the power spectrum. Figure 4 depicts the typical spectra for wakefulness, nonREM sleep and REM sleep.

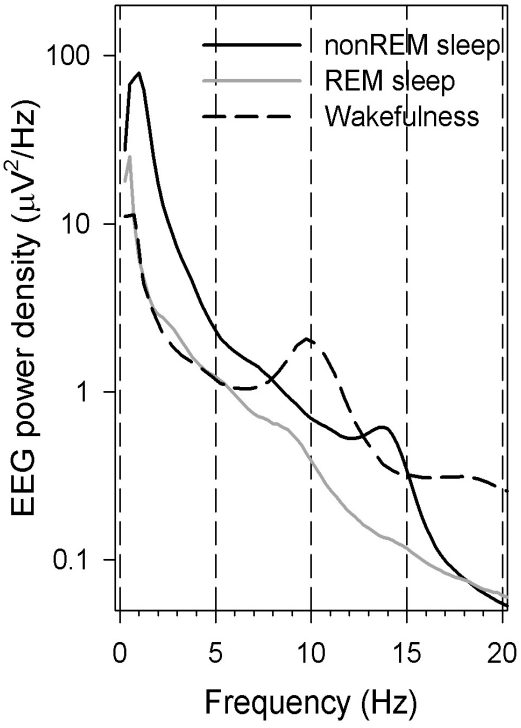

Figure 4: Power spectra of wakefulness, nonREM sleep and REM sleep
Mean EEG power spectra of rested wakefulness with eyes open (C3A2 derivation): Average over four 5-min EEG recordings with open eyes conducted at 08:15, 11:00, 14:00 and 17:00 after a baseline night (12 men and 10 women). Frequency resolution: 0.5 Hz.

Mean EEG power spectra of nonREM sleep (stages 2-4) and REM sleep (C3A2 derivation): Mean EEG power spectra of an 8-hour baseline night (12 men and 10 women). Frequency resolution: 0.25 Hz.

1.4.1. Characteristics of the Waking EEG

The scalp EEG in wakefulness is characterized by low voltage, mixed frequency EEG (Fig. 5A) It is generally subdivided into several frequency bands for detailed analysis. These bands are: delta (< 3.5 Hz), theta (4 - 7.5 Hz), alpha (8 – 13 Hz), beta (14 – 30 Hz), and gamma (> 30 Hz). However, it should be noted that the frequency bands, especially the lower and upper limits of the bands, are variable and differ considerable in the literature. The alpha rhythm, reflecting a peak in the EEG spectrum at around 10 Hz, characterizes rested wakefulness with eyes closed. As soon as subjects open their eyes or when they pay attention the alpha peak attenuates (Berger, 1929, Klimesch, 1999). Distinct features of the alpha peak in rested

wakefulness are genetically determined (Bodenmann et al., 2009a). The peak is also suggested to reflect diseases, as for example Alzheimer's disease (Hassainia et al., 1997) or Parkinson's disease (Kamei et al., 2010) and environmental influences during wakefulness, as for example radio frequency electromagnetic fields (Regel et al., 2007).

1.4.2. Characteristics of the nonREM and REM Sleep EEG

Based on the criteria established by Rechtschaffen and Kales (1968) nonREM sleep is further subdivided into 4 stages. More specifically, stage 1 reflects the transition from wakefulness to sleep onset. It is an intermediate state between wakefulness and sleep, characterized by an irregular low-voltage, mixed frequency EEG. Stage 1 progresses into nonREM sleep stage 2. The most prominent features in stage 2 are K-complexes and/or sleep spindles (Fig. 5B). The K-complex consists of a brief negative sharp wave immediately followed by a slower positive component, with a duration of at least half a second, whereas the sleep spindle consists of ~ 12 – 14 Hz waves that occur for at least 0.5 seconds and recur approximately every 3 – 10 seconds. They show a characteristic shape with progressively increasing than decreasing amplitude. Usually, stage 2 sleep reflects rather superficial nonREM sleep and the tonic EMG is generally low. NonREM stages 3 and 4 contain moderate (20 – 50 %) and large amounts (> 50 %) of EEG delta waves (~ 0.5 – 4.5 Hz) a correlate of SWA with high-amplitude (> 75 μV peak to peak), which are generally referred to as SWS (Fig. 5C). Sleep spindles may or may not be present in SWS and muscle tone is generally low.

The characterization of REM sleep EEG is similar to that of stage 1, showing low-amplitude and high-frequency waves (Fig. 5D). However, during REM sleep, muscle tone is low and rapid eye movements (REM) are present. In this state, autonomic nervous activity increases variability in heart rate and blood pressure (Somers et al., 1993).

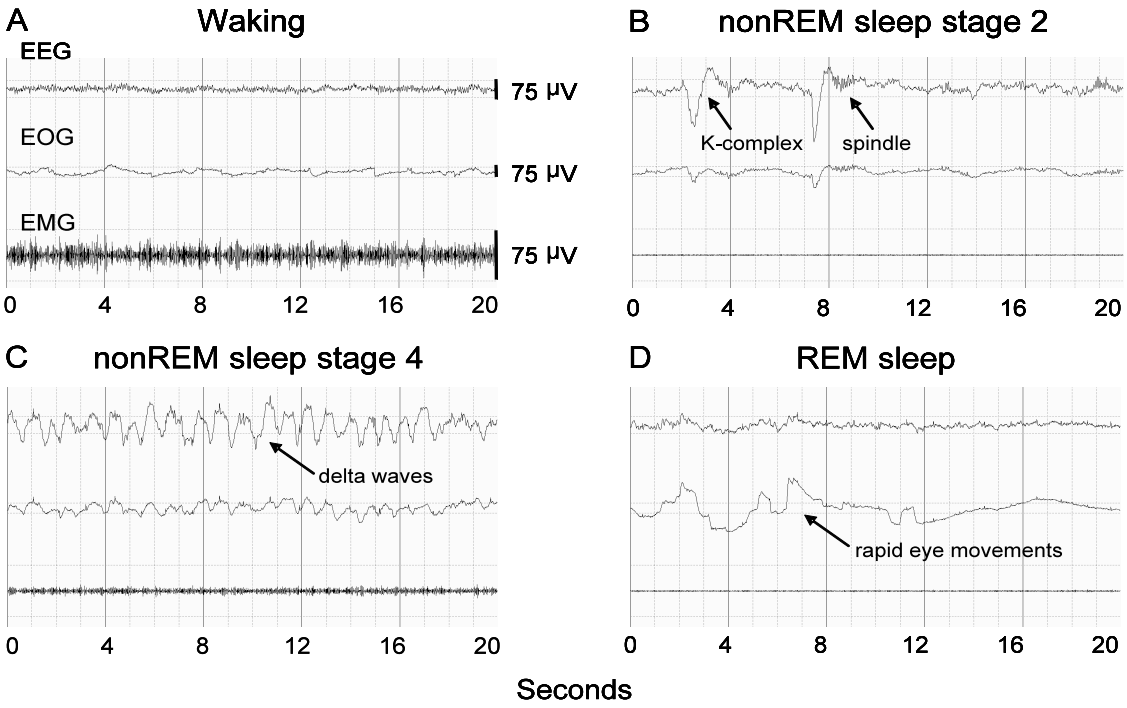

Figure 5: Characteristic EEG, EOG and EMG signals in waking and sleep
(A) The waking state is characterized by alpha activity (~ 8 – 13 Hz), and/or low voltage, mixed frequency EEG and generally, high muscle tone. **(B)** Stage 2 of nonREM sleep includes two transient events, namely K-complexes and sleep spindles (~11 – 15 Hz) on the background of a low-voltage mixed frequency EEG. **(C)** Deep nonREM sleep (stages 3 and 4: Slow wave sleep (SWS)) is dominated by EEG delta waves with a frequency of ~ 0.5 – 4.5 Hz. **(D)** REM sleep shows low voltage, mixed-frequency EEG, rapid eye movements (REM) and muscle atonia.

In humans, the continuous recording of sleep shows that nonREM sleep and REM sleep states alternate throughout the night (Aserinsky and Kleitman, 1953, Dement and Kleitman, 1957, Borbély and Achermann, 2005). A normal 8-hours sleep episode consists of four or more consecutive nonREM/REM sleep cycles with a period length of roughly 90 to 120 minutes (Fig. 6, top panel). Moreover, the proportion of different sleep stages in a sleep cycle differs across the sleep episode. The percentage of REM sleep and stage 2 sleep increases during the course of the night, whereas the amplitude of SWA is highest at the beginning of the sleep episode and decreases from the first to the last sleep cycle (Borbély, 1982, Borbély and Achermann, 2005). The decrease of SWA across the night corresponds with the dissipation of homeostatic sleep pressure (Fig. 6, middle panel), which can be identified in the color-coded power spectra (Fig. 6, bottom panel) by the presence of warm colors.

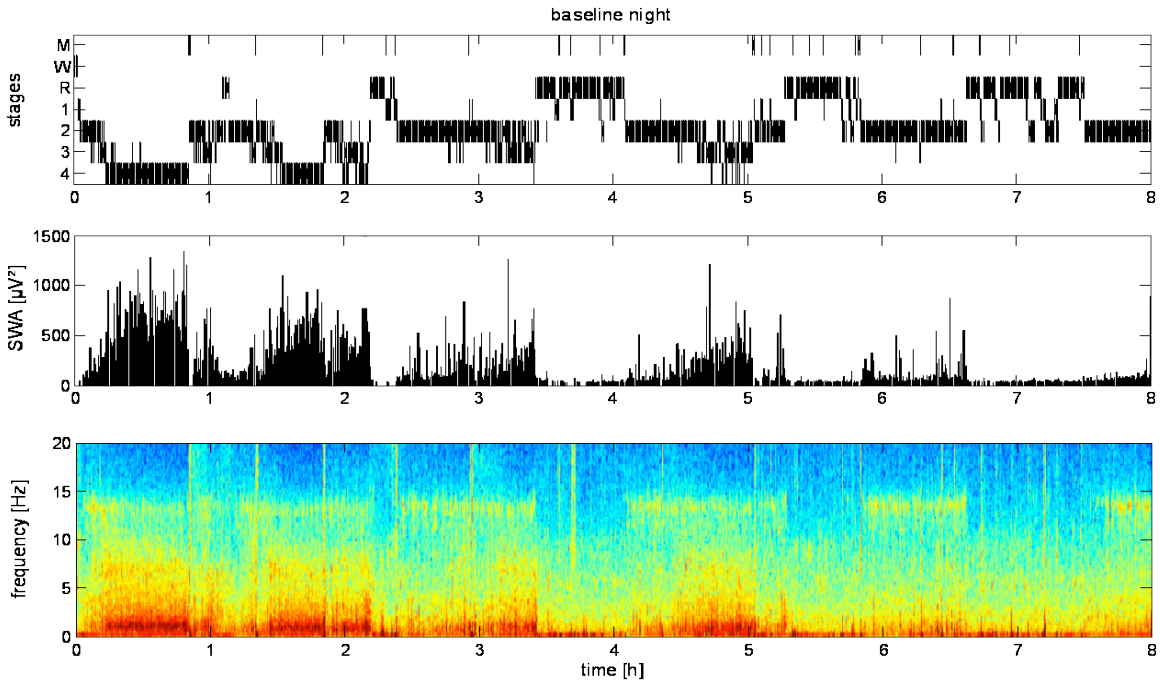

Figure 6: Sleep profile (hypnogram), slow-wave activity (SWA, 0.5-4.5 Hz) and color-coded power spectra of consecutive 20-second epochs (average of 5 spectra calculated for 4-s epochs; Hanning window) during an 8-hours nocturnal sleep-episode
Upper panel: Movement time (M), Waking (W), REM sleep (R) and nonREM sleep (stages 1 to 4). Middle panel: Time course of SWA. Epochs scored as 20-s epochs. M and W were excluded. Lower panel: Spectra (0 – 20 Hz) are color coded on a logarithmic scale
(0 dB = 1μV²/Hz; -10 dB  20 dB).

1.5. Brain mechanisms that control sleep and wakefulness

The most important difference between the waking brain and the sleeping brain is the state of consciousness. While awake we are aware of and responsive to the environment and as soon as we fall asleep, we are largely unresponsive to external stimuli. Thus, the physiological state of sleep is mainly characterized by reversible unconsciousness, strong reduced responsiveness to external stimuli and immobile posture.

It was first observed by von Economo (1876 - 1931) that patients affected with the viral infection *Encephalitis lethargica* exhibited excessive sleepiness (Von Economo, 1930). Von Economo found that these patients had lesions at the junction of the midbrain and the diencephalon. This was in contrast to some other patients of von Economo's, which were unable to fall asleep. The reason for the sleeplessness in these patients was a damaged anterior hypothalamus. Based on these findings, he speculated that sleep and waking are active processes, each controlled by specific structures of the brain. The assumption was

further investigated by Bremer (1935), who uncovered evidence of an ascending arousal system (AAS) responsible for sleep-wake regulation. More than a decade later, Moruzzi and Magoun (1949) provided further evidence for the concept of an AAS.

In summary, the brain circuits and neurons, which are involved in the regulation of sleep and wakefulness are localized mainly in the brainstem, hypothalamus and basal forebrain (BF) and project via the thalamus to the cortex (Saper et al., 2001, Hobson and Pace-Schott, 2002, Pace-Schott and Hobson, 2002, Steriade, 2003, Fort et al., 2009). Thus, it is evident that wakefulness, nonREM sleep and REM sleep are regulated at many levels and that the simultaneous and compensatory contribution of multiple neuronal and neurotransmitter systems is important for physiological sleep-wake functioning.

1.5.1. Wakefulness: neuronal structures and pathways of arousal

The AAS is important for maintaining wakefulness and generating REM sleep. It originates in the brainstem and BF and runs through the midbrain reticular formation (RF) activating the thalamus and cortex (Moruzzi and Magoun, 1949, Siegel, 2004b, Jones, 2005a, Saper et al., 2005). The AAS consists of two pathways (Fig. 7): The first pathway is the dorsal pathway, which ascends to the thalamus, activating the thalamic relay neurons, which in turn transmit information to the cortex. These cells are mainly cholinergic (ACh) and originate in the upper region of the brainstem, in the nuclei of the pedunculopontine tegmentum (PPT) and of the laterodorsal tegmentum (LDT) (Hallanger et al., 1987, Jones and Beaudet, 1987) (Fig. 7, yellow pathway). Due to this input, thalamocortical transmission is facilitated. In addition, glutamatergic neurons of the RF are also important in arousal and maintaining the waking state (Siegel, 2004a, Jones, 2005b). The second pathway, the ventral pathway, bypasses the thalamus and originates in the upper brainstem and caudal hypothalamus and projects to the lateral hypothalamic area, the BF and the cerebral cortex (Saper et al., 2005). This pathway is comprised not only of cholinergic neurons from the RF, but also of monoaminergic cell groups. These monoaminergic projections contain noradrenaline (NA) from the locus coeruleus (LC), serotonin (5-HT) from the dorsal raphe nuclei, dopamine (DA) from the ventral periaqueductal gray matter (vPAG), and histamine (His) from the tuberomammillary nucleus (TMN). The ventral pathway also includes the neuropeptide hypocretin (Hcrt), whose cell bodies are located in the lateral hypothalamus, playing an important role in the stabilization of wakefulness and sleep (Adamantidis et al., 2007,

Sakurai, 2007). Hypocretin is also known as orexin. Deficiencies in Hcrt neurotransmission, has it has been shown in animal studies, causes the sleep disorder narcolepsy (Lin et al., 1999, Mochizuki et al., 2004). This was further supported from studies in narcoleptic patients with reports of low or undetectable levels of Hcrt in the cerebrospinal fluid (Nishino et al., 2000, Mignot et al., 2002) and from postmortem studies in human brain tissues, which reported cell loss in Hcrt-containing brain regions (Thannickal et al., 2000).

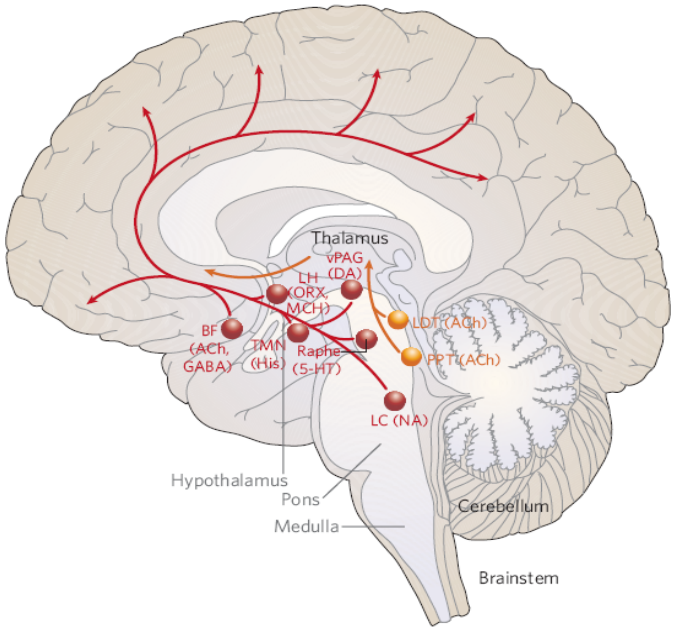

Figure 7: The Ascending Arousal System (AAS) (from Saper et al., 2005)
Schematic representation of some key components of the ASS. Wakefulness is maintained by cholinergic (Acetylcholin: ACh) ascending inputs from the upper pons, the pedunculopontine (PPT) and the laterodorsal temental nuclei (LDT) to the thalamus, which in turns facilitates thalamocortical transmission (yellow pathway). A second pathway (red) contributes to the waking cortical activation by inputs of monoaminergic cell groups, including the tuberomammillary nucleus (TMN) containing histamine (His), the cell groups of the periaqueductal gray matter (vPAG) containing dopamine (DA), the raphe nuclei containing serotonin (5-HT) and the locus coeruleus (LC) containing noradrenaline (NA). This pathway also receives contributions from peptidergic neurons in the lateral hypothalamus containing orexin (ORX) or melanin-concentrating hormone (MCH), and from basal forebrain (BF) neurons that contain γ-amino-butyric-acid (GABA) or ACh.

1.5.2. NonREM sleep: neuronal mechanisms

The ventrolateral preoptic area (VLPO) is situated above the optic chiasm. Neurons of the VLPO, which contain the inhibitory neurotransmitters γ-amino-butyric-acid (GABA) and galanin, inhibit the arousal-producing areas and promote sleep (Fig. 8). As a consequence, primarily the neurons in the hypothalamus and brainstem that belong to the AAS (Sherin et al., 1996, Gaus et al., 2002, Saper et al., 2005) are inhibited, which in turn inhibit the VLPO

neurons by their neurotransmitters during wakefulness. This mechanism leads to a reciprocal interaction of wake-promoting and sleep-promoting neurons across the sleep-wake cycle (Luppi, 2010) and prevents intermediate states between sleep and wakefulness.

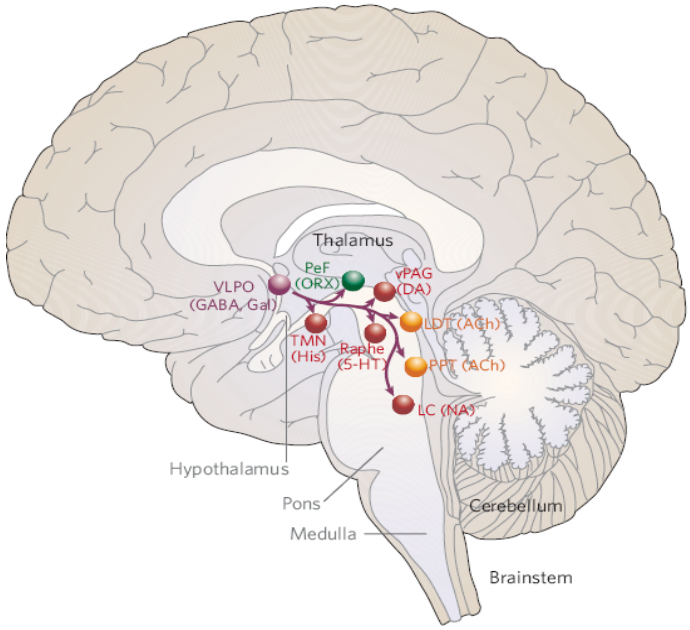

Figure 8: The ventrolateral preoptic area (VLPO) projections to components of the ascending arousal system (AAS) (from Saper et al., 2005).
Schematic representation of the key projections of the ventrolateral preoptic area (VLPO) to the main components of the AAS. It includes inhibitory inputs (γ-amino-butryic-acid (GABA) and Galanin (Gal)) to monoamiergic cell groups (red), such as the tuberomammillary nucleus (TMN), the cell groups of the periaqueductal gray matter (vPAG), the raphe cell groups and the locus coeruleus (LC). Additionally, the VLPO innervates neurons in the lateral hypothalamus (green), including the perifornical (PeF) orexin (ORX) neurons, and interneurons in the cholinergic (Acetylcholin: ACh) cell groups (yellow), the pedunculopontine (PPT) and laterodorsal temental nuclei (LDT). 5-HT: Serotonin, NA: Noradreanline, His: Histamine.

It is proposed that the homeostatic regulation of sleep may involve chemical factors, which promote sleep after they have accumulated during wakefulness (Krueger et al., 2008). On the contrary, inhibition of these sleep factors reduces sleep need and sleep rebound after sleep deprivation. A primary endogenous sleep factor to contribute to the homeostatic regulation of sleep is the neuromodulator adenosine. Adenosine is formed throughout the nervous system by the metabolic breakdown of adenosine-triphosphate (ATP) (Latini and Pedata, 2001). The role for adenosine as proposed endogenous sleep-promoting substance will be discussed in chapter 1.6.1.

1.5.3. REM sleep: neuronal mechanisms

During REM sleep cortical activity closely resembles that of wakefulness. In parallel, occular movements, autonomic activation and loss of muscle tone are characteristics of REM sleep. Similar to wakefulness, the ACh-containing neurons in the PPT/LDT system and BF fire most frequently in REM sleep (Sinton and McCarley, 2004). In contrast to the activated cholinergic neurons, the monoaminergic neurons, especially those from the LC containing NA and those from the raphe nuclei containing 5-HT cease firing in REM sleep (Hobson et al., 1975, McGinty and Harper, 1976, Aston-Jones and Bloom, 1981). Thus, it is proposed that "REM-off" monoaminergic neurons inhibit the REM-promoting, "REM-on" neurons (McCarley and Massaquoi, 1992, Siegel, 2004a, Sinton and McCarley, 2004, Saper et al., 2005). This reciprocal interaction is important for the generation of the ultradian rhythm of REM sleep. The muscle atonia, observed during REM sleep is controlled by glutamatergic and glycinergic projections to the spinal cord (Siegel, 2004b).

1.5.4. The thalamocortical system and the cortical EEG

The overall activity in the talamocortical (ThCx) system is thought to be regulated by the AAS and it has been shown that thalamic relay neurons fire in patterns that correlate with cortical EEG recordings (Steriade et al., 1993a). Thus, the waking and REM sleep EEG show fast electrical activity, because the wake-promoting neurons are depolarized and discharge in a tonic, single-spike mode, which is reflected in the EEG as desynchronized activity. At the transition from wakefulness to nonREM sleep, neuronal activity changes dramatically. This change is reflected in the EEG, which displays increased amounts of large-amplitude, low-frequency oscillations. These changes are associated with a reduced firing of the neurons in the AAS. Additionally, signals from the outside world are no longer transmitted to the cerebral cortex. As a consequence, ThCx neurons exhibit prolonged periods of hyperpolarisation and show an increase in membrane conductance (low threshold Ca^{2+} spikes and increased potassium conductance). These changes result in reduced membrane potentials and allow ThCx neurons to generate synchronized, rhythmic activity characterized by a burst-pause firing mode. Due to the interaction between ThCx neurons, cortical neurons and reticular thalamus neurons, three oscillations are prominent during nonREM sleep: Spindles (11 – 15 Hz), delta waves (0.5 – 4.5 Hz) and the slow oscillation (< 1 Hz).

Sleep spindles are generated in GABAergic thalamic reticular neurons that impose rhythmic inhibitory sequences onto these neurons. The generation of these rhythmic inhibitory postsynaptic potentials is possible when the membrane potential in these cells is around - 60 mV, which allows burst-pause firing spindle oscillations, which are subsequently transferred to the cortex (Steriade et al., 1990). With the deepening of nonREM sleep, a further hyperpolarisation of the ThCx relay neurons, below -70 mV occurs and sleep spindle oscillations are replaced by delta waves (Steriade et al., 1991).

Delta waves are the main source of SWA, the established EEG marker of sleep homeostasis, and exhibit a declining trend in the course of a night (Fig. 6). In contrast to the network which generates spindle oscillations, the delta oscillations reflect intrinsic oscillations of single ThCx cells resulting from the interplay between intrinsic thalamic cell-membrane currents (McCormick and Pape, 1990). Additionally, delta waves are generated in the cortex (Steriade et al., 1993b).

The slow oscillation is considered to be different from SWA. The slow oscillation does not exhibit the typical decline of power from the first to the second nonREM sleep episode as it is found for SWA (Achermann and Borbély, 1997, Amzica and Steriade, 1997). The slow oscillation originates from intracortical networks and is comprised of a depolarization phase, the so called up-state, where neurons fire at high rates followed by a long-lasting hyperpolarized phase, so called down-state, where neurons are silent (Steriade et al., 1993c, Steriade, 2003). One function of the slow oscillation is to group other sleep oscillations, as thalamic sleep spindles (Fig. 9A), thalamic clock-like delta waves (Fig. 9B), and cortical delta waves (Fig. 9C) (Contreras and Steriade, 1995, 1996, Amzica and Steriade, 2002, Steriade, 2003).

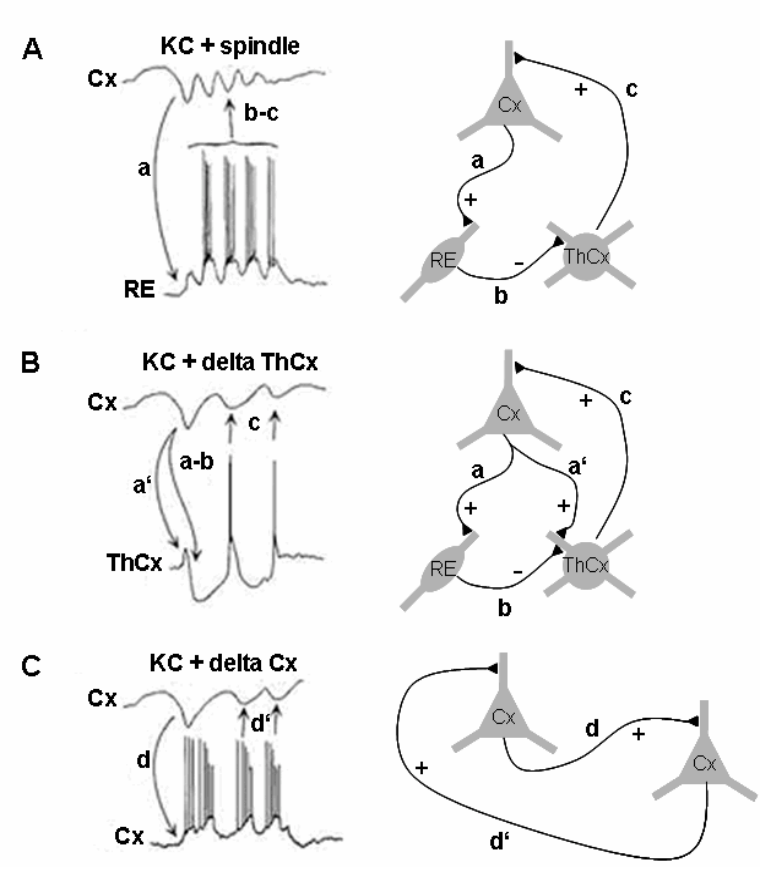

Figure 9: Coalescence of the depolarizing phase of the slow oscillation (K-complex (KC)) with other rhythms. (from Amzica and Steriade, 2002)
Left side: Membrane potentials and intracellular recordings are represented. **Right side:** Schemes of the circuits involved in the generation of the respective EEG patterns are illustrated. The synaptic projections are indicated with small letters, corresponding to the arrow at left, which indicate the time sequence for the events.
(+): Excitatory postsynaptic potentials (EPSPs); (-): inhibitory postsynaptic potentials (IPSPs). **(A)** Combination of a KC with a spindle sequence. A KC in the cortex (Cx) travels through the corticothalamic pathway (a) and triggers in thalamicreticular neurons (RE) a spindle sequence that is transferred to the thalamocortical cells (ThCx) of the dorsal thalamus (b) and thereafter back to the cortex (c) where it shapes the tail of the KC.
(**B**) Modulation of a KC by a sequence of clock-like delta waves originating in the thalamus. The KC travels along the corticothalamic pathway (a') eliciting an EPSP curtailed by an IPSP produce along the cortico-RE (a) and RE-ThCx (b) projections. The hyperpolarisation of the thalamocortical cells generates a sequence of low-threshold potentials crowned by high-frequency bursts of spikes at delta frequency that reach the cortex through the thalamocortical link (c).
(**C**) Modulation of a KC by a sequence of delta waves originating in the cortex. When the KC impinges upon bursting cells (d) it triggers a series of rhythmic bursts of spikes at delta frequency that may have a greater impact on target cells (d') than single action potentials, thus synchronizing several neurons whose membrane potentials will be reflected in local field potentials as delta waves.

1.6. Genetic influences on cognition and sleep-wake regulation

It is widely accepted that genes contribute to brain plasticity and thus, to variations in cognitive performance (Egan et al., 2001, Egan et al., 2003, Hariri et al., 2003, Dempster et al., 2005, Savitz et al., 2006). Moreover, individual differences in brain activation patterns during cognitive performance might depend to some extend on genetic variation (Duncan et al., 2000, Koten et al., 2009).

The synaptic homeostasis hypothesis postulates that synaptic potentiation during wakefulness and synaptic downscaling during sleep is important to keep the overall synaptic

weight in balance (Tononi and Cirelli, 2003, 2006). Thus, sleep is beneficial for brain plasticity. Direct evidence supporting the net increase in synaptic weight onto neurons occurring during wakefulness comes form the findings that molecular changes are associated with LTP. LPT induces expression of plasticity-related genes such as Arc, BDNF or the P-CREB protein (Cirelli and Tononi, 2000). The net increase of LTP-related genes expression increases further if wakefulness is prolonged and decreases as soon sleep is occuring. Thus, habitual sleep duration might be important for synaptic potentiation during wakefulness and synaptic downscaling during sleep. Interestingly, it was recently shown in adolescences that habitual short sleep duration is inversely associated with measures of IQ (Geiger et al., 2010). Taken into account the synaptic homeostasis hypothesis, this would mean that shorter sleep duration permits additional time for synaptic potentiation during wakefulness, when compared to longer sleep duration. In addition, whether an individual is a habitual short or long sleeper is in part genetically determined (He et al., 2009, Allebrandt et al., 2010). For example, a short sleep phenotype in humans was recently associated with a point mutation in a protein of the helix-loop-helix family affecting transcription of the hDEC2-P385R gene (He et al., 2009). In addition, a human CLOCK gene variant was suggested to be associated to longer sleep duration in humans (Allebrandt et al., 2010). Intriguingly, another polymorphism on the CLOCK gene was associated with diurnal preferences in humans (Katzenberg et al., 1998, Mishima et al., 2005).

Moreover, the scalp EEG, used to gain insights into sleep-wake regulation, is one of the most heritable traits in humans (van Beijsterveldt et al., 1996, Finelli et al., 2001a, Buckelmüller et al., 2006, Tang et al., 2007, Tafti, 2009). Spectral analysis on waking EEG recordings in twins showed very similar patterns in all EEG frequency bands and in all brain regions tested (van Beijsterveldt et al., 1996). Furthermore, spectral analysis on sleep EEG in baseline and recovery sleep after sleep loss revealed that the heritability in the frequency range between 8 and 16 Hz is roughly 96 % (Ambrosius et al., 2008, De Gennaro et al., 2008). Thus, the EEG provides a unique fingerprint in the regulation of sleep in humans. Further evidence for trait-like individual differences in the sleep EEG comes from studies in healthy humans, showing high intra-individual stability in nonREM sleep EEG power in the frequency range of 0.75 – 17 Hz, whereas inter-individual variability was highest in the frequency band between 5 and 15 Hz (Buckelmüller et al., 2006). Notably, the inter-individual variability in the nonREM sleep spectra was even more pronounced than the average response to sleep deprivation (Finelli

et al., 2001a, Tucker et al., 2007). On the basis of these studies, it is evident that the sleep and waking EEG consist of systematic and stable inter-individual differences (Tucker et al., 2007) that are at least in part genetically determined (Landolt, 2008). To study candidate genes is one possibility to understand better the molecular mechanisms underlying sleep-wake regulation.

In humans for example, there exists the functional p.Val66Met polymorphism of *BDNF*, which modulates working memory functions (Egan et al., 2003, Savitz et al., 2006) and has also been shown to be involved in neurodegenerative disorders (Brunoni et al., 2008, Zuccato and Cattaneo, 2009). Generally, BDNF is not only an established mediator of synaptic plasticity, but it may be causally related to the homeostatic regulation of sleep (Huber et al., 2007, Faraguna et al., 2008). Studies of the *BDNF* genotype on human sleep are still lacking. Therefore, we examined as part of this dissertation, whether the *BDNF* polymorphism has an impact on sleep homeostasis in healthy humans (chapter 4).

Moreover, it has been shown that BDNF-induced modulation of glutamate release and synaptic plasticity requires the activation of adenosine A_{2A} receptors, which consequently influences TrkB receptor function, the main target of BDNF to induce LTP (Assaife-Lopes et al., 2010). Interestingly, the stimulant caffeine, which acts primarily as a competitive antagonist at A_1 and A_{2A} receptors in central nervous system, prevents sleep loss-induced deficits in LTP (Alhaider et al., 2010). Therefore, BDNF and adenosine may interact to mediate the consequences of neural activity during wakefulness on sleep. In addition, endogenous adenosine and its receptors play also an important role in sleep-wake regulatory mechanisms (Basheer et al., 2004, Landolt, 2008b). Our group previously showed that the p.Asp8Asn polymorphism of adenosine deaminase (*ADA*) predicts differences in deep slow-wave sleep and sleep intensity in healthy young men in baseline night (Rétey et al., 2005). Therefore, we were interested whether the polymorphism of *ADA* interferes with sleep homeostasis and neurobehavioral performance during prolonged wakefulness (chapter 5).

Plastic synaptic processes are not only modulated by genetic polymorphisms of *BDNF* and *ADA* but also by the p.Val158Met polymorphism of *COMT,* which modulates declarative (de Frias et al., 2004) and working memory processes in adults (Egan et al., 2001, Savitz et al., 2006). It also predicts differences in EEG alpha power between 11 and 13 Hz in all vigilance states (Bodenmann et al., 2009a). Thus, BDNF (Huber et al., 2007, Faraguna et al., 2008),

adenosine (Landolt, 2008b) and catecholamines (Monti, 1982, Wisor and Eriksson, 2005) provide possible neurochemical mechanisms to link synaptic plasticity occurring during wakefulness and homeostatically regulated sleep need. Apart from synaptic plasticity, it has been shown that a variable number tandem repeat (VNTR) polymorphism in the coding region of the circadian clock gene, *PER3*, modifies sleep and waking EEG markers in humans (Archer et al., 2003, Viola et al., 2007). Individuals carrying the longer allele ($PER^{5/5}$) have more SWS and delta activity in nonREM sleep, higher theta/alpha activity in REM sleep and wakefulness if compared to individuals carrying the shorter allele ($PER^{4/4}$). Thus, the *PER3* polymorphism has been interpreted to reflect differences in the homeostatic accumulation of sleep pressure.

In summary, these studies show that the different aspects of cognitive functions and sleep-wake regulatory processes are under the control of a multitude of genes and depend to some extent on similar neuronal mechanisms (Andretic et al., 2008, Koten et al., 2009, Landolt, 2008). Most intriguingly, single genes can profoundly affect cognitive functions and sleep-wake regulatory processes and contribute to trait-like inter-subject variation in both, cognition and sleep (Andretic et al., 2008, Landolt, 2008a).

1.6.1. Main metabolic pathway of BDNF and its role in sleep-wake regulation

BDNF acts mainly in the central nervous system, where it is involved in the growth and differentiation of neurons and synapses. Additionally, BDNF is an established mediator of synaptic plasticity, widely distributed in the hippocampus, cortex and BF. These areas are involved in learning, memory and executive functioning (Yamada and Nabeshima, 2003, Waterhouse and Xu, 2009). BDNF belongs to the family of neurotrophins and binds to two membrane receptors, namely to the high affinity receptor tyrosine kinase (TrkB) and to the low affinity receptor pan neurotrophin receptor ($p75^{NTR}$) (Carvalho et al., 2008, Cunha et al., 2010). BDNF is synthesized as a precursor protein, the pre-proBDNF (Barker, 2009), which has its pre-sequence cleaved off in the endoplasmic reticulum (ER). The pro-region or N-terminal fragment is either proteolytically cleaved before secretion of BDNF or by extracellular proteases, such as plasmin, after the secretion of proBDNF into the extracellular space.

There exist two main pathways that process BDNF (Fig. 10): The constitutive secretory pathway, which releases BDNF as proBDNF and the regulated pathway, which releases the

mature BDNF (mBDNF) in an activity and Ca^{2+} dependent manner (Lu, 2003). Once released, proBDNF binds preferentially to the $p75^{NTR}$ receptor and mBDNF binds particularly to TrkB receptors. The activity-dependent secretion of mBDNF is involved in cell survival and synaptic plasticity and in the regulation of LTP (Nagappan and Lu, 2005, Waterhouse and Xu, 2009). This is in contrast to the interaction of $p75^{NTR}$ with proBDNF, which induces apoptosis and long-term depression. Thus, interaction with different receptors reveals different biological functions.

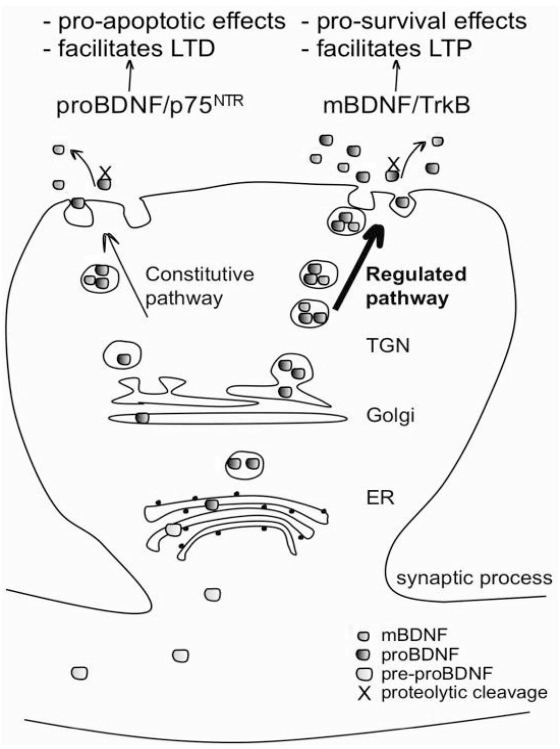

Figure 10: BDNF processing, packaging and secretion in neurons (from Cunha et al., 2010)
BDNF is synthesized as a pre-proBDNF protein, which has its pre-sequence cleaved off in the endoplasmic reticulum (ER). The resulting proBDNF moves, via the Golgi apparatus, into the trans-Golgi network where two kinds of secretory vesicles are generated: those of the constitutive secretory pathway and those of the regulated pathway, whose secretion is activity-dependent. ProBDNF packaged in both types of vesicles is either proteolytically cleaved and secreted as mature BDNF (mBDNF), or secreted as proBDNF and cleaved by extracellular proteases. Once released, proBDNF binds preferentially to pan neurotrophin receptor ($p75^{NTR}$) and mBDNF binds preferentially to both pre- and post- synaptic tyrosine kinase B (TrkB) receptors.

The importance of the pro-region of BDNF was highlighted by the finding that a single nucleotide polymorphism (SNP) in the pro-region of the protein has an impact on synaptic targeting and the activity-dependent secretion of BDNF (Egan et al., 2003). The polymorphism leads to an amino acid change at codon 66 in the 5' pro-region of the human BDNF gene, which affects cell trafficking and packing (Egan et al., 2003). This valine (Val) to methionine (Met) conversion has been associated with impaired hippocampal activity and memory functions as well as with neurodegenerative disorders (Zuccato and Cattaneo, 2009).

In relation to sleep-wake regulation it has been shown that BDNF expression during wakefulness was predicted by the amount of exploratory behaviour in rats (Huber et al.,

2007). This study also found that high expression of BDNF influenced the homeostatic response of SWA during subsequent sleep. Moreover, it has been shown that the amount of BDNF in rodent cortical areas (barrel cortex) is positively correlated with SWA during subsequent sleep (Faraguna et al., 2008). Thus, it appears that BDNF plays a causal role in the homeostatic regulation of sleep. In this dissertation, we investigated for the first time in healthy adults, whether the p.Val66Met polymorphism of *BDNF* affects sleep-wake regulation (chapter 4).

1.6.2. Main metabolic pathways of adenosine and its role in sleep-wake regulation

The ubiquitous purine nucleoside adenosine and its receptors are involved in many physiological processes. In the central nervous system adenosine acts as an inhibitory neuromodulator modulating the release of neurotransmitters. On the other hand, adenosine acts as a homeostatic regulator of energy metabolism in the brain. Increased energy demand enhances the need for ATP, which in turn increases extracellular adenosine concentrations. Thus, extracellular adenosine is a by-product of energy metabolism. Under normal physiological conditions extracellular adenosine concentration are low, because of the relatively high activity of adenosine kinase (AK), which converts adenosine into adenosine-monophosphate (AMP) in a reversible way (Fig. 11). In addition, adenosine is irreversibly deaminated into inosine by ADA. Deamination of adenosine into inosine is important, especially if extracellular adenosine concentrations are high. Adenosine is formed from AMP intracellularly by 5'-nucleotidase (5'-N) or extracellulary by 5'-ectonucleotidase (5'-EN). Clearance of extracellular adenosine occurs through specific nucleoside transporters of adenosine (AT) (reviewed in Fredholm et al. (2005a), Landolt (2008b), Latini & Pedata (2001)). The effects of adenosine are mediated via four subtypes of G-protein-coupled metabotropic receptors: A_1, A_{2A}, A_{2B}, A_3. Adenosine shows the highest binding potency for the A_1 receptor (Schwierin et al., 1996, Basheer et al., 2007, Elmenhorst et al., 2007) and A_{2A} receptor (Urade et al., 2003, Hayaishi et al., 2004). In the brain, A_1 receptors are expressed in the cortex, thalamus, hippocampus and basal ganglia, whereas A_{2A} receptors are present at high concentrations in the striatum, nucleus accumbens and olfactory bulb (Fredholm et al., 2005a, Landolt, 2008b). By contrast, the expression of A_{2B} receptors seems to be low in the cerebral cortex and A_3 receptors appear to play an inferior role in the central nervous system (Fredholm et al., 2000, Yaar et al., 2005).

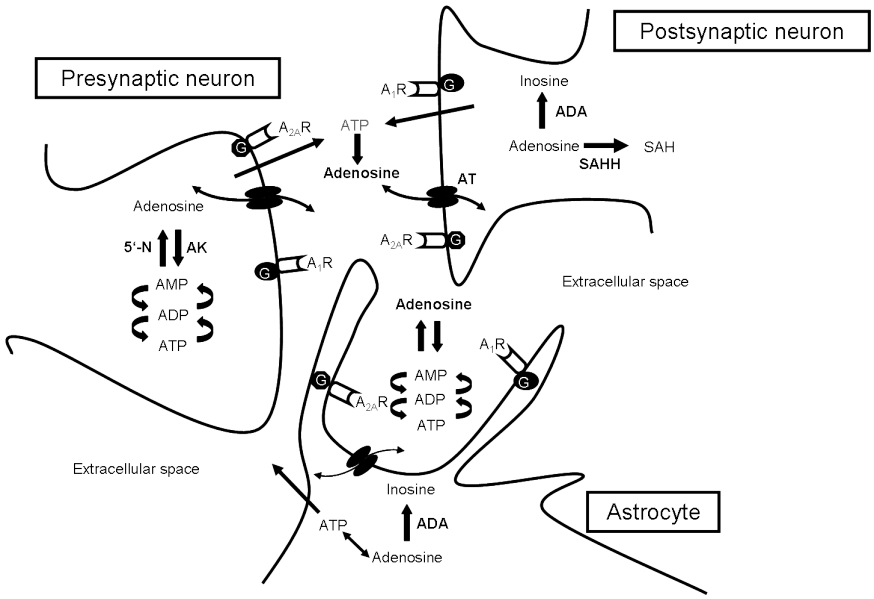

Figure 11: Simplified schematic representation of the main metabolic pathways of adenosine
AMP: adenosine-monophosphate; **ADP**: adenosine-diphosphate; **ATP**: adenosine-triphosphate; **A_1R**: adenosine A_1 receptor; **A_{2A}R**: adenosine A_{2A} receptor; **G**: G-protein coupled receptor; **ADA**: adenosine deaminase; **AK**: adenosine kinase; **AT**: nucleoside transporters of adenosine; **5'-EN**: 5'-ectonucleotidase; **5'-N**: 5'-nucleotidase; **SAH**: S-adenosyl-homocysteine; **SAHH**: S-adenosyl-homocysteine hydrolase

Adenosine may play an important role in the homeostatic regulation of energy in the brain during the sleep-wake cycle (Benington and Heller, 1995, Illes et al., 2000). This hypothesis was first proposed by Benington & Heller (1995), which states that glycogen depletion during wakefulness leads to an increase in extracellular adenosine, which promotes sleep. Recently the energy hypothesis of sleep was revisited and it was proposed that in addition to the metabolites adenosine and glycogen, other pathways, as for example the unfolded protein response are involved in the catabolic mechanisms during wakefulness and the anabolic mechanisms during sleep (Scharf et al., 2008). Nevertheless, neuronal activity during wakefulness provides an energy challenge to the brain, and a proposed important function of sleep is to refill brain glucose stores. That endogenous adenosine is an important sleep promoting factor, was shown in microdialysis studies in rats and cats, where they observed a rise in extracellular adenosine levels during wakefulness and a decline during sleep in the BF and the cortex (Porkka-Heiskanen et al., 1997, Porkka-Heiskanen et al., 2000). In contrast, adenosine levels in other brain areas as for example in the thalamus, preoptic area of the hypothalamus or the dorsal raphe nucleus remained the same or decreased.

Studies in humans also support the notion that adenosine plays an important role in sleep homeostasis (Landolt, 2008b). Strong support for this assumption comes from the fact that caffeine, a popular stimulant of the central nervous system, acts as an competitive, non-selective antagonist at the A_1 and A_{2A} adenosine receptors and promotes wakefulness (Fredholm et al., 1999). Furthermore, self-rated sensitivity to the wake promoting effects of caffeine depends on the genotype of the c.1976T>C single nucleotide polymorphism of *ADORA2A* in healthy students (Rétey et al., 2007). With respect to the EEG, caffeine intake attenuates theta activity (5-8 Hz) in waking and SWA (0.75 – 2 Hz) in nonREM sleep (Landolt et al., 1995, Landolt et al., 2004). Based on our previous findings that the p.Asp8Asn polymorphism of *ADA* predicts difference in SWS and SWA (0.5 – 5.75 Hz) in baseline sleep (Rétey et al., 2005), we wanted to further elucidate the role for the *ADA* polymorphism on human sleep-wake regulation and neurobehavioral performance during prolonged wakefulness (chapter 5).

1.7. Objectives and structure of the thesis

The primary aim of this thesis was to investigate the association between cognitive abilities, habitual sleep and sleep-wake regulation. We investigated whether distinct genetic polymorphisms contribute to individual differences in neurobehavioral performance in baseline and sleep-deprived states.

In the first study, I systematically tested cognitive abilities in 118 women and 127 men to assess executive functions, attention, learning and memory. With this large data set obtained, including measures reflecting different cognitive domains, I examined whether the interaction of number and space relies on prefrontal cortex contribution (chapter 2). More specifically, I studied whether implicit asymmetric orientation along a mental number line towards small numbers, as measured by the RNG, is associated with hemispheric lateralization of prefrontal executive functions, as measured by design and letter fluency tests.

In view of possible relationships between cognition and sleep duration, as suggested in children and adolescents (Jenni et al., 2009, Geiger et al., 2010), I also addressed the question whether executive functioning and habitual sleep duration are associated in healthy adults (chapter 3).

To investigate genetic factors contributing to sleep homeostasis, I studied the impact of two distinct polymorphisms on sleep and the sleep EEG in healthy individuals. For the study reported in chapter 4, I investigated in 22 healthy subjects according to a matched-pair design, whether sleep-wake regulation is affected by the p.Val66Met polymorphism of *BDNF*. To this end, I examined whether this polymorphism affects waking performance during prolonged wakefulness and sleep homeostasis. In a similar approach the impact of the p.Asp8Asn polymorphism of *ADA* on sleep homeostasis was studied in 22 subjects. (chapter 5). The results of all studies will be summarized in a synopsis and discussed in chapter 6.

Chapter 2

Asymmetric prefrontal cortex functions predict asymmetries in number space

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Abstract

Little is known about the neuropsychological factors that contribute to individual differences in the asymmetric orientation along the mental number line. The present study documents healthy subjects' preference for small numbers over large numbers in a random number generation task. This preference, referred to as "small number bias" (SNB), varied with prefrontal functional lateralization: it was larger in participants with over-proportionately better performance in design fluency compared to letter fluency than in participants with over-proportionately better performance in letter fluency when compared to design fluency. Asymmetries in learning and memory tasks (verbal vs. non-verbal) were not related to direction or size of the SNB. We conclude that hemispheric asymmetries of specifically prefrontal executive functions are predictive of an individual's lateral orientation bias along the mental number line. Therefore, the focus on parietal contributions to spatial-numerical associations may not be justified. Random number generation may be a helpful method to further explore these associations uncontaminated by the asymmetric involvement of response effectors.

Keywords:
Functional hemispheric asymmetry, number processing, random number generation, prefrontal cortex, executive functions, spatial attention

Introduction

In a seminal paper, Dehaene et al. (1993) described a surprising relationship between numbers and space. In a parity decision paradigm with bimanual responses, low numbers (e.g.. 1 or 2) of a given range were faster classified with the left hand, whereas larger numbers (e.g., 8 or 9) were consistently faster responded to with the right hand. This phenomenon is referred to as the Spatial Numerical Association of Response Codes (SNARC) effect and provides support for the notion that numbers are cognitively represented along a spatially oriented "mental number line" [for a recent meta-analytic review of the SNARC literature see Wood et al. (2008)]. The brain mechanisms of SNARC are still not fully understood. One of the experiments in Dehaene et al.'s (1993) original study found that, when subjects had their hands crossed during responding, there was still a right (left) side advantage for large (small) numbers. This finding suggested that the SNARC effect did not reflect a hemispheric specialization for small or large numbers *per se,* but a more abstract stimulus-response compatibility effect in the representation of number magnitude. However, the latter study by Wood et al. (2006) did not report evidence of a SNARC with crossed hands. These conflicting findings might result from the fact that Wood et al.'s subjects had already performed on a SNARC paradigm without their hands crossed before they repeated the experiment with their hands crossed. Under these circumstances, the hands-crossed condition revealed no SNARC effect, suggesting that hand-based associations may well play a role in number-hemispace, and thus number-hemisphere associations. Against this background and in the light of recent findings pertaining to the cortical involvement in number processing (Wood and Fischer, 2008), it may be worth revisiting the issue of functional hemispheric asymmetries in the emergence of an asymmetric exploration of number space.

According to the influential triple-code model of number representation, number concepts are represented bilaterally in the parietal lobes (Dehaene, 1997), and bilateral parietal cortex probably plays a crucial role in numerical cognition (Hubbard et al., 2005). Support for this view comes from several studies. For example, addition and subtraction operations are linked to neural circuits in the bilateral posterior parietal lobules that also represent right- and left-directed eye movements (Knops et al., 2009). Arithmetic operations can thus be likened to movements, both of the eyes and of the attentional focus, along the mental

number line (Pinhas and Fischer, 2008, Loetscher et al., 2010). Moreover, patients suffering from left spatial neglect can misplace the midpoint of a numerical intervals to the right of the real midpoint (Zorzi et al., 2002, Zorzi et al., 2006). The impression that these patients ignore the left sides of both their physical and their mental space has lent further support to the view that numbers are obligatorily projected onto space through some process relying on parietal cortex.

Compared to parietal contributions, frontal cortical contributions to numerical cognition have been somewhat overlooked, although several recent studies suggest that they may be especially important. For example, Doricchi et al. (2005) pointed out that neglect in physical and mental space can be dissociated and that numerical biases were associated with specifically prefrontal damage. Furthermore, single cell recordings in non-human primates show more number-specific neural activity in frontal areas compared to parietal areas (Nieder, 2009, Bongard and Nieder, 2010). Moreover, children comparing numerical values invoke the same network of brain regions as adults do, including frontal, parietal, and occipital areas. However, during numerical tasks, they recruit inferior frontal cortex to a much greater degree than adults do (Kaufmann et al., 2006, Cantlon et al., 2009). Finally, random number generation (RNG) relies on dorsolateral prefrontal cortex functions (Jahanshahi et al., 2000, Gottselig et al., 2006). Several neuroimaging studies showed that frontal and parietal cortex, as well as subcortical areas are involved in RNG tasks (Itagaki et al., 1995, Daniels et al., 2003). More recently, the method of RNG was proposed as a possible method for the investigation of asymmetries in number space (Loetscher and Brugger, 2007).

In the present study we wish to document prefrontal cortex contributions to numerical cognition. Specifically, we propose that healthy adults' cognitive lateralization profile predicts individual differences in attending to the left or right side of the mental number line. From experiments in RNG it is known that the frequency of small numbers exceeds that of large numbers (Dehaene, 1997, Loetscher and Brugger, 2007). The causes for this "small-number bias" (SNB) are unclear, yet several possible explanations are conceivable. For example, small numbers may simply be overrepresented because they are learned earlier in life (Dehaene, 1997), or because they are more frequently used (Dehaene and Mehler, 1992). Alternatively, the concept of “pseudoneglect in number space” (Oliveri et al., 2004, Gobel et al., 2006, 2006) explains the SNB via spatial-attentional processes. Thus, SNB could be a

consequence of a cerebral hemispheric imbalance in favour of right-hemisphere spatial-attentional functions. Support for the latter account of SNB comes from experiments during which human subjects were required to turn their head laterally while generating numbers "at random" (Loetscher et al., 2008). Compared to a baseline condition (facing straight ahead), significantly more small numbers were generated when facing left, and more large numbers when facing right. In another experiment, the size of the SNB in RNG depended on the activation of left or right hemispheric processes in a dual task. Specifically, SNB was diminished when subjects were simultaneously engaged in a letter fluency task, but significantly enhanced, when subjects generated random numbers while performing on a concurrent design fluency task (Loetscher and Brugger, 2007). Together, these findings suggest that directing one's attention along the number line can be manipulated by the same variables that reportedly shift one's attention in physical space. In particular, as illustrated by the dual-task RNG results of Loetscher and Brugger (2007), activation of left hemisphere prefrontal cortex functions appears to draw one's attention towards larger numbers, whereas activation of right anterior cortical regions is accompanied by an exaggerated SNB.

While these previous findings reflect experimentally induced, state-dependent shifts of hemispatial attention, the present experiment aimed at investigating trait-like asymmetries in the magnitude of a person's orientation in number space. By "state" we refer to a person's momentary orientation bias (determined, e.g., by the nature of the task, test conditions etc.). By "trait" we refer to an individual's inclination, over and above any state variables, to orient more to the left than to the right side, or to have more verbal compared to nonverbal abilities. We required healthy right-handers to perform a RNG task and predicted that individual SNB would be associated with performance in two fluency tasks, one relying predominantly on right prefrontal functions (design fluency) and the other on left-hemisphere prefrontal functions (letter fluency). Our hypothesis was that a more pronounced SNB (corresponding to a marked pseudoneglect along the number line) would be linked to an individual's superiority for right-hemisphere mediated design fluency relative to left-hemisphere-mediated letter fluency. To control for asymmetric contributions of learning and recall to SNB (arguably reflecting more temporal lobe than prefrontal mediation), verbal and non-verbal learning and memory tests were performed.

Methods

Participants

A total of 209 healthy adults (102 women, 107 men), aged between 18 and 40 years (mean age ± S.D.: 25.2 ± 5.3 years) with no history of psychiatric or neurological disorders participated in the study. All participants were right-handed according to a 13-item inventory (Chapman and Chapman, 1987). They had recently completed or were still engaged in their education. The native language of all participants was German. Testing was performed according to the ethical standards of the Declaration of Helsinki. All participants provided written informed consent prior to the experiment.

Tasks

Random number generation (RNG):

We used the Mental Dice Task that requires the oral production of the digits from 1 to 6 in a sequence "as random as possible" (Loetscher and Brugger, 2007). This task is intuitive and easy to comply with for naïve healthy participants as well as for most patients. Number generation was paced by a 1 Hz metronome, and a total of 66 random numbers were produced.

Letter fluency:

During three minutes, participants named as many words as possible beginning with the letter "S". Proper names, variations and repetitions were not allowed. This task taps left hemisphere prefrontal executive functions (Perret, 1974, Ravnkilde et al., 2002).

Design fluency:

During three minutes, participants drew as many straight-line connections as possible among sets of five dots each. The response sheet contained 40 dot matrices, identical to the five-dot arrangement on a dice. Pattern repetitions were not allowed. Originally developed by Regard et al. (1982), this task taps right hemisphere frontal executive functions (Lezak, 1983, Ruff et al., 1994, Baldo et al., 2001).

Verbal and non-verbal learning and memory:

To control for asymmetric contributions of learning and memory to the SNB in RNG, corresponding tests were performed. Verbal and non-verbal abilities were assessed by the Rey Auditory Verbal Learning Test (RAVLT) and the Rey Visual Design Learning Test (RVDLT).

Specifically, subjects completed on both tasks five learning trials, one immediate recall trial and one delayed recall trial (1 hour after immediate recall) (Foster et al., 2009). Learning and memory reflect temporal lobe functioning to a more pronounced degree than do fluency tasks.

Data analysis

To control for response stereotypy, a measure of zero-order redundancy and a RNG-index reflecting redundancy on the level of pair frequencies were calculated for each participant's number sequence (Towse and Neil, 1998). SNB in RNG was calculated as the difference between the total occurrence of small numbers (1, 2, 3) and the total occurrence of large numbers (4, 5, 6). Positive values reflect a bias to name more small numbers than large numbers (Loetscher and Brugger, 2007).

To obtain a measure of relative proficiency of left and right frontal functioning, we computed for each participant the difference in the number of items produced in the design fluency task minus the number of items produced in the letter fluency task and divided this difference by the sum of produced items. This fluency index ranged from -0.28 to +0.57, with a median value of 0.11. A median split resulted in 104 participants (44 women; 60 men) with relatively better performance on the design fluency task (= DESIGN fluency group, henceforth) and 105 participants (58 women, 47 men) with relatively better performance on the letter fluency task (= LETTER fluency group)[1].

To receive a measure of the relative ability to learn and remember, respectively, verbal and nonverbal information, we calculated for each participant three performance indices by computing the difference between RAVLT and RVDLT for learning, immediate recall and late recall and divided these differences by the sum of produced items. These indices reflect relative superiority in the verbal or non-verbal domains. We then calculated median split values for these three performance indices. The index for the learning performance ranged from -0.47 to +0.26, with a median value of 0.00. The index for immediate recall performance ranged from –0.41 to +0.33, with a median value of 0.03. Finally, the index for delayed recall performance ranged from –0.41 to +0.33, with a median value of 0.03. Each index resulted in two median-split groups (relatively higher scores compared to relatively

[1] Four subjects showed a value of 0.11. They were included in the LETTER fluency group in order to have a most comparable number of participants in both groups

lower scores in the particular tasks) that were compared with respect to the magnitude of SNB. For the index of learning performance 100 subjects (45 women; 55 men) were compared to 109 subjects (58 women; 51 men), for the index of immediate recall performance 109 subjects (47 women; 62 men) were compared to 100 subjects (56 women; 44 men) and for the index of delayed recall performance 114 subjects (50 women; 64 men) were compared to 95 (53 women; 42 men) subjects, respectively.

Statistics

All statistical analyses were performed using SAS 9.1.3 software (SAS Institute, Cary, NC). The significance level was set at $\alpha < 0.05$. To assess potential group differences in stereotypy on the RNG task and to control for gender differences in the SNB, fluency index, design fluency index and letter fluency index, we performed two-tailed, unpaired *t*-tests. The overall SNB score was tested against zero with a dependent one-sample *t*-test. To calculate group differences on SNB between the DESIGN fluency group and LETTER fluency group, an analysis of covariance (ANCOVA) was conducted with "age" and "years of education" as covariates. Furthermore, a Pearson's product-moment correlation coefficient was calculated between SNB and fluency index. Analogous two-tailed, unpaired *t*-tests were calculated for the control tasks that is, for the median-split groups based on the performance differences in RAVLT and RVDLT.

Results

Analysis of response stereotypy revealed no differences between the DESIGN fluency group and LETTER fluency group with respect to redundancy ($t(207) = 1.4$, $p > 0.1$) and RNG index ($t(207) = -1.0$, $p > 0.3$), respectively.

There was no difference between women and men in SNB ($t(208) = 1.6$, $p > 0.1$), in the fluency index ($t(208) = 1.2$, $p > 0.2$), in the design fluency test ($t(208) = 0.2$, $p > 0.8$) and in the letter fluency test ($t(208) = 1.5$, $p > 0.1$). Furthermore, despite the fact that, numerically, there were more women (58) than men (47) in the LETTER fluency group and more men (60) than women (44) in the DESIGN fluency group, the corresponding Chi-square analysis was not significant (Chi-Square = 3.00, p = 0.083).

As expected, the overall SNB was significantly different from zero, $t(208) = 3.4$, $p < 0.001$. More specifically, numbers 1 to 3 were named on average 33.7. ± 0.2 times, whereas

numbers 4 to 6 were named on average only 32.3 ± 0.2 times (Fig. 1). Note that if a real die were rolled 66 times, the expected frequency of each number would be 11, with small (1, 2, 3) and large (4, 5, 6) numbers occurring with a frequency of 33.

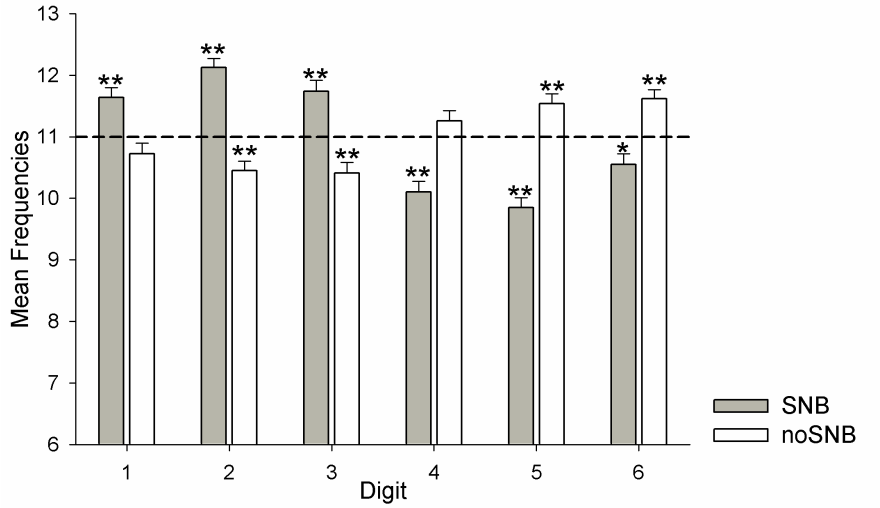

Figure 1: Mean frequencies of the single digits in the Mental Dice Task
Mean frequencies of the single digits (± standard errors of the mean) in the Mental Dice Task (generation of 66 random numbers). The horizontal line shows the expected frequency (=11) for a real die, rolled 66 times. Asterisks indicate significant differences between observed and expected frequencies (** $p < 0.001$, * $p < 0.01$, one sample *t*-test). Gray bars: subjects (n=107) with a small number bias (SNB) larger than the median value of 0 for the entire population (n=209). White bars: subjects with a smaller-than-median SNB (n=102).

The ANCOVA revealed a main effect of fluency group ($F(1, 205) = 4.5$ $p < 0.035$). Thus, participants of the DESIGN fluency group (better performance in design fluency compared to letter fluency) named 34.0 ± 0.3 small numbers, whereas participants of the LETTER fluency group (relatively better letter fluency) named 33.2 ± 0.2 small numbers (Fig. 2). By contrast, the covariates age ($F(1, 205) = 3.1$, $p > 0.07$) and years of education ($F(1, 205) = 0.0$, $p > 0.86$) had no significant influence. For the group as a whole, individual SNB in RNG correlated with the fluency index ($r=0.2$, $p < 0.01$), suggesting a small (Cohen's $d = 0.33$) but significant association between SNB and a relative right hemisphere superiority.

Analyses of the control indices of mnestic functioning revealed no systematic association with SNB. Thus, SNB did not differ between median-split groups based on performance indices in learning, immediate or late recall on RAVLT and RVDLT ($t(207) < 1.4$, $p > 0.1$). Likewise, correlations between SNB and the different indices were not significant ($0 < r < 0.04$, $p > 0.6$).

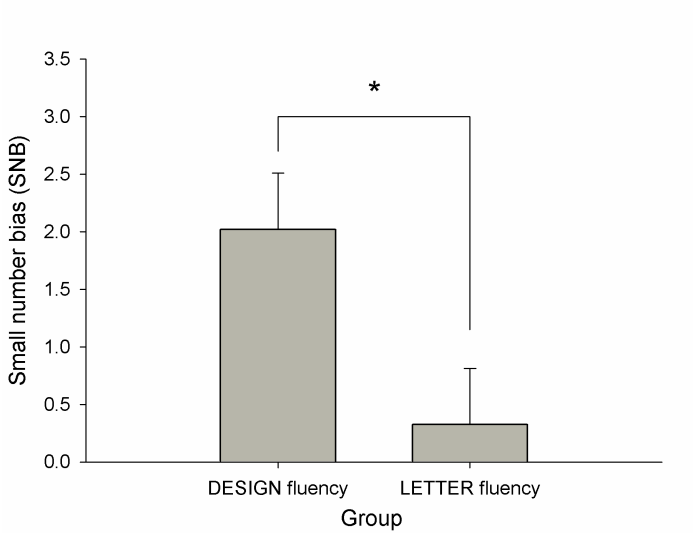

Figure 2: Small number bias (SNB) in the participant groups with relatively better design fluency than letter fluency
SNB (difference between the total occurrence of numbers 1,2, and 3 and the total occurrence of numbers 4,5 and 6 in the Mental Dice Task) in the participant groups with relatively better design fluency than letter fluency (DESIGN fluency group, n=104) and that with relatively better letter fluency than design fluency (LETTER fluency group, n=105). Superiority in design fluency (mediated by *right* prefrontal cortex) is associated with larger SNB ($p < 0.04$). Error bars represent standard errors of the mean.

Discussion

While generating the digits from 1 to 6 "at random" (a RNG task), healthy right-handed subjects produced more small (1, 2, and 3) than large (4, 5, and 6) numbers. This finding confirms the robustness of a "small number bias" (SNB) in RNG tasks with different sampling ranges, (Loetscher and Brugger, 2007, Brugger et al., 2010), as well as in number guessing experiments (Ertel, 2005). Using a large sample size helped us to document a relatively small (Cohen's d = 0.33), but systematic difference in this pervasive bias between subjects differing in the lateralization of prefrontal functions as a trait.

What is the nature of this SNB? Different causes were proposed, and these fall largely into two classes, non-spatial and spatial explanations. Let us first consider the non-spatial accounts of SNB. One such account is the suggestion that small numbers are preferred over larger numbers because they are learned earlier in life and may therefore be more saliently represented in memory, and more readily available during random number retrieval (Dehaene, 1997). Acquisition of the counting sequence begins with the smallest numbers at around age three and proceeds until about age seven when children understand "how to

count principles" (Gelman and Gallistel, 1978) and can construct infinitely large numbers. Another non-spatial account points out the fact that, in multi-digit numbers taken from any source of measurement, the frequency of the leading digit decreases with increasing magnitude. For example, by crushing blocks of a certain mineral, Kreiner (2003) found that the leading digit in the weights (in g) of more than 11,000 resulting pieces decreased steadily from 1 to 9, with respective occurrences of 5312, 2303, 1345, 908, 614, 426, 355, 286, 234. This so-called Benford-Newcomb law (Newcomb, 1881, Benford, 1938) was recently discussed as a potential source of SNB in man-made number strings [data fabrication: Diekmann (2007); numerical guessing: Burns (2009)]. It arguably reflects the ability of human subjects to pick up statistical regularities in numerical measurement and to structure their own numerical output accordingly when asked to produce number strings. In connection with the SNARC effect, the Benfod-Newcomb law was previously mentioned by Fischer et al. (2010).

In contrast to these non-spatial accounts of SNB, the notion of an asymmetric availability of points on the mental number line predicts that small numbers benefit from an attentional advantage over larger numbers. This advantage is proposed to be equivalent to the one enjoyed by left-sided items in a horizontal arrangement of visually or haptically presented stimuli [Bradshaw, Nathan, Nettleton, Wilson, & Pierson (1987): rod centering] and referred to, alternatively, as "initial exploration asymmetry" (Hättig, 1992, Ebersbach et al., 1996), "left-side underestimation" (Bradshaw et al., 1983), "right hemispatial inattention" (Weintraub and Mesulam, 1988) or "pseudoneglect" (Bowers and Heilman, 1980). Pseudoneglect along the mental number line was originally demonstrated in the bisection of numerical intervals (Oliveri et al., 2004). Its magnitude is sometimes influenced by the same factors that also determine the magnitude of attentional asymmetries in physical space (Longo and Lourenco, 2007), but Doricchi et al. did not find a relationship between visual and number line bisection (Doricchi et al., 2009). The preferred evocation of small numbers in RNG was recently proposed to be a manifestation of pseudoneglect (Loetscher and Brugger, 2007). In a series of experiments, these authors showed that individual differences in pseudoneglect in spatial tasks were reflected in corresponding differences in the magnitude of the SNB in RNG. For instance, those subjects judging the emotional expression of happy/sad chimeric faces predominantly according to the *left*-sided expression also produced over-proportionately many small numbers in the Mental Dice Task (randomizing the digits from one to six). The predominant judgement of left- or right-sided emotional

expression is a stable individual characteristic (Yovel et al., 2008). In another experiment, the magnitude of SNB in a RNG task was decreased by a simultaneous verbal fluency task, but increased during the concurrent generation of drawings (Loetscher and Brugger, 2007). While in that experiment verbal and design fluency were used as state variables of differential hemispheric arousal, the present study investigated a relative preference for one of these two fluency tasks *as a trait*, in the sense described in our Introduction. To this end, participants performed the Mental Dice Task, a letter fluency task and a design fluency tasks. Corroborating previous work with these two fluency tasks (Brugger et al., 1996a), we found that subjects generated more figures than words, perhaps indicating that the design fluency task may have been easier than the letter fluency task. The fluency index we used, however, provided a measure of functional asymmetry uncontaminated by task difficulty. Interestingly, task performance was independent of gender and the frequency distribution of women and men within the two fluency groups was balanced. Therefore, our findings are not confounded by gender. As predicted, we found that those subjects, who showed an above-average advantage for design fluency over letter fluency, also exhibited significantly more pronounced SNB in the Mental Dice Task. This finding indicates that trait-like behavioral asymmetries over prefrontal sites may co-determine the strength of healthy subjects' orientation along the mental number line and thus predict the magnitude of pseudoneglect. Because our age span of participating subjects was quite large (18-40 years) and subjects were recruited with different educational backgrounds we performed a covariance analysis with these two factors. While education proved unrelated to direction and magnitude of SNB, older subjects showed a tendency towards a higher SNB. This tendency is in line with the prediction of an increase in the SNARC with increasing age due to decreased inhibitory control (Wood et al., 2008). Asymmetries in mnestic functions (learning and recall, less mediated by frontal cortex compared to fluency) were entirely unrelated to the preference for small numbers.

The present result is also in line with the observation that, in hemispatial neglect, specifically right frontal lesions are accompanied by a neglect for small numbers (Doricchi et al., 2005). Moreover, it may explain why, using the Mental Dice Task, we had been unable to demonstrate a large-number bias in a population of neglect patients (Loetscher and Brugger, 2009). In that study, a majority of patients had cortical lesions either confined to the parietal lobes or at parieto-frontal sites of the right hemisphere; only a small minority

had left-sided neglect after fronto-temporal or even exclusively frontal (n=1) lesions (Loetscher and Brugger, 2009). However, the fact that transcranial magnetic stimulation (TMS) over the right parietal cortex can simulate neglect-like biases in the numerical midpoint estimates of healthy subjects (Göbel et al., 2006) indicates that the importance of frontal asymmetries may be limited to numerical tasks with implicit spatial components. Unlike the midpoint estimation of number intervals, RNG does not require direct access and exploration of the mental number line. Dissociations between explicit and implicit measures of asymmetries in number space have previously been noted (Priftis et al., 2006, Loetscher and Brugger, 2009). They also matter in SNARC paradigms, where dual task experiments revealed a disruption of space-number interactions by verbal load during parity decisions (i.e. an implicit evocation of spatial codes), but a similar disruption by spatial load during magnitude decisions [i.e. when a more explicit use of spatial codes is required: van Dijck, Gevers, & Fias (2009)]. However, the response requirements of a standard SNARC paradigm do not allow disentangling interference effects on the level of stimulus processing from those on the level of lateralized response execution. Future research should study hemisphere-specific interference effects in manual response paradigms as well as in tasks requiring verbal responses. In both settings, implicit ways of using magnitude information should be distinguished from explicit ways. Furthermore, trait and state variables of hemispheric asymmetry should be considered to foster our current understanding of the role of left and right hemispheres for the emergence of asymmetries in number space. Finally, as pointed out by a reviewer, a potential influence of handedness on asymmetries in number space could be investigated. Although absent in the SNARC paradigm (Dehaene et al., 1993), marked interactions between handedness and hemispatial processing were recently described (Casasanto, 2009).

In summary, the present study documents individual differences in the extent of healthy subjects' preference for small numbers over large numbers. These differences are associated with hemispheric differences in specifically prefrontal executive functions. We conjecture that, in addition to the role of the parietal lobes, prefrontal cortex contributions to asymmetries in number space should be considered in future research. Finally, random number generation may provide a means to overcome the asymmetric involvement of response effectors in existing methods for the assessment of associations between numbers and space.

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Chapter 3

Performance on the Stroop Color-Word interference task is negatively associated with habitual sleep duration in healthy adults

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Introduction

Recent research addressed the question whether sleep duration and higher cognitive functions may be associated in non-sleep-deprived individuals. A longitudinal study in a sample of 493 healthy children followed from birth to late adolescence (7 to 16 years of age) showed a consistent negative relationship between habitual sleep duration (based on questionnaires) and intelligence quotient (IQ) (Jenni et al., 2009). Recently, the same group examined more systematically whether sleep duration and intelligence scores are associated in healthy children (7.5 to 11.2 years) (Geiger et al., 2010). The authors assessed habitual sleep duration by questionnaires and recorded seven or more days of actigraphy data, an objective estimate of habitual sleep length. They confirmed that the shorter the sleep duration the higher the IQ scores in these children. Nevertheless, the negative correlation was only valid for self-reported sleep duration during weekends and for fluid IQ measures. In contrast, a study in healthy school-age children showed that short sleep duration was associated with poorer performance on measures of overall IQ (Gruber et al., 2010). Other studies suggest that sleep is essential for the adequate development of cognitive functions and learning during childhood (Gomez et al., 2006, Backhaus et al., 2008, Nixon et al., 2008).
There exist only a few studies that explore the relationship between habitual sleep duration and cognitive abilities in middle-aged adults (Kronholm et al., 2009, Mograss et al., 2010). One of these studies revealed a U-shaped association between habitual sleep duration and cognitive functioning, indicating that short (6 hours or less) and long sleep duration (9 hours or more) are associated with decreased verbal fluency, as well as encoding and retaining of verbal material (Kronholm et al., 2009). However, associations were small ($r=0.2 – 0.4$) due to high interindividual variability. In older adults (60 years and over) confirmed that cognitive performance, as assessed by a modified version of the Mini-Mental State Examination (Folstein et al., 1975), was inversely associated with sleep duration. However, in a similar approach short sleep duration was not related to cognitive functions, as measured by the Mini-Examen Cognoscitivo (MEC) (Faubel et al., 2009). The inconsistent findings may result from the fact that the methods used to quantify habitual sleep duration and higher cognitive performance differed between the studies. In addition, data were often limited to self-reported sleep duration and objective estimations of sleep-wake patterns by actigraphy were lacking. Finally, when actigraphy data were available, recordings only lasted a few

days, which renders the interpretation of the data difficult. Moreover, the relationships between individual sleep duration and higher cognitive abilities are complex and further research is needed. In addition, habitual sleep duration varies greatly among healthy individuals with an average sleep duration of roughly 7.5 hours a night (Borbély, 1984, Groeger et al., 2004), and a standard deviation of approximately 1 hour (Webb, 1979). There exist few cases of extreme short or long sleepers. For example, a famous short sleeper is Michelangelo, who reported sleeping only four hours a night. These individuals exhibit short natural sleep duration in the absence of any daytime sleepiness, which is in contrast to excessive daytime sleepiness, indicating insufficient sleep. On the other hand, famous long sleepers have been also reported, as for example Einstein, who spent up to ten hours in bed. The question arises: what are the underlying physiological mechanisms responsible for these variable habitual sleep durations in healthy individuals? The neurobiological basis for the interindividual variability in biological sleep duration is not clear. Neurophysiological studies revealed that physiological short sleepers (habitual sleep duration < 6 hours) may tolerate higher homeostatic "sleep pressure" than do physiological longer sleepers (habitual sleep duration > 9 hours) (Aeschbach et al., 1996, Aeschbach et al., 2001). More specifically, the prolongation of wakefulness enhanced SWA, a marker of sleep intensity, significantly more in long sleepers than in short sleepers, whereas the dynamics of sleep homeostasis was similar in both groups (Aeschbach et al., 1996). Based on the synaptic homeostasis hypothesis (Tononi and Cirelli, 2003, 2006) and with studies showing a close relationship between sleep, local brain plasticity and cognitive processes (Huber et al., 2004, Huber et al., 2006), we hypothesized that better tolerance of high sleep pressure, as exhibited in short sleepers (Aeschbach et al., 2000, Aeschbach et al., 2001) would permit an increased time for the acquisition of cognitive processes. As a consequence, better cognitive performance would be associated with shorter sleep duration.

We aimed to examine more systematically whether a relationship between habitual sleep duration and performance on distinct cognitive abilities exists in healthy, non-sleep-deprived, young (25.2±0.4 years) adults. We focused on distinct executive functions, such as planning, updating of information, inhibition, creativity, decision-making and reasoning. Whether and which of these functions are sensitive to short-term total sleep deprivation is highly debated (Harrison et al., 2000, Jones and Harrison, 2001, Sagaspe et al., 2003). For instance, word fluency (Harrison et al., 2000) and the Stroop task (McCarthy and Waters,

1997) have been shown to be sensitive to sleep deprivation. However, some studies show opposite findings on these two tasks (Binks et al., 1999). For consecutive and more reliable quantification of habitual sleep duration, self-reported and objective measures were combined. Sleep duration was estimated based on validated questionnaires (n=220) and 4-week wrist-actigraphy and a sleep diary (n=82). Executive functions were individually assessed by use of distinct neuropsychological tasks. Attention, learning and memory were also quantified.

Measures

Participants

A total of 220 subjects were studied (115 men, 105 women; age: 25.2 ± 0.4 years). All subjects were right handed, in good health, exhibited normal body weight (BMI: 22.1 ± 0.2) and reported not taking central-nervous-system affecting medication on a regular basis. All subjects had recently completed or were still engaged in some higher education.

Assessment of higher cognitive functions

Executive functioning, attention, learning and memory were tested in a 2-hour session. To optimize performance subjects were scheduled at their preferred testing time, if possible. Because we focused on executive functioning, several distinct executive abilities were assessed: The Stroop Color-Word Task (Stroop, 1935) to quantify inhibition-interference control, a letter (Perret, 1974) and design fluency test (Regard et al., 1982) to quantify verbal and nonverbal fluency, a random number generation (RNG) test for the assessment of counting and stereotypy (Loetscher and Brugger, 2007) and the Go/No-Go test to measure response inhibition (Greenwald et al., 1998). Attention was quantified by the d2 attention task (Brickenkamp, 1962). The Rey Auditory Verbal Learning Test (RAVLT), the Rey Visual Design Learning Test (RVDLT) (Strauss et al., 2006, Foster et al., 2009), and the digit span test (Miller, 1956) were administered to test learning and memory. Depending on performance on each task, subjects were divided by median split of the main test variables into higher- and lower-functioning individuals (for an overview see Table 1). Habitual sleep duration on workdays and on leisure days was then compared between the two groups.

Table 1: Dependent on median-split (Q_2), individuals were divided into higher- and lower-functioning performers

Outcome Variables	Q_1	Q_2	Q_3	n - Higher		n - Lower	
				F	M	F	M
Stroop Color-Word task:							
Interference/Inhibition score (sec)	4	6	8	58	53	47	62
Random number generation test:							
Redundancy	0.4	0.6	1.1	56	57	49	58
Response stereotypy (adjacency)	32	38	43	46	61	59	54
Go/No-Go task:							
Reaction time (ms)	387	415	445	48	56	53	56
Nonverbal fluency test (5 point test):							
Sum of admissible figures designed	39	44	51	50	54	55	61
Verbal fluency test (s word test):							
Sum of admissible words produced	25	35	41	55	48	50	67
D2 attention task:							
Number of processed items	487	538	582	60	51	45	64
Sum of omission and commission errors	462	509	554	60	49	45	66
Attention variability	9	11	14	55	57	50	57
Rey Auditory Verbal Learning Test (RAVLT):							
Number of words recalled (five trials)	49	56	62	57	49	48	66
Rey Visual Design Learning Test (RVDLT):							
Number of figures recalled (five trials)	51	56	62	49	56	56	59

Depending on performance on each task, subjects were divided by median split into higher- (n - Higher) and lower- (n - Lower) functioning performers (n=220). Q_1 = 25th percentile, Q_2 = 50th percentile, Q_3 = 75th percentile, F: Female, M: Male.

To control for daytime sleepiness between higher- and lower-functioning performers, the Epworth Sleepiness Scale (ESS) (Johns, 1991), where subjects rate the probability of falling asleep in even various every-day situations were administered before testing. In addition, education level (years) was verified to exclude that better performance in some individuals is associated with better education of these individuals. Finally, preferred testing time and actual testing time within individuals were assessed and compared between higher- and lower-functioning performers, to control for whether cognitive performance in some participants might have been influenced by more or less appropriate testing times.

Habitual sleep duration

All subjects filled in the Munich Chronotype Questionnaire (MCTQ) (Roenneberg et al., 2003) to estimate subjective sleep duration during work and leisure (i.e., weekend) days. To verify rest-activity patterns more objectively, 4-week wrist actigraphy (AW: wrist-worn Actiwatch©, Cambridge Neurotechnology Ltd, Cambridge, UK) was collected in 38 women and 50 men,

while living at home in their habitual social environment. Actigraphy records physical activity based on an accelerator inside the monitor. This method provides a non-invasive, reliable and objective estimation of sleep-wake patterns (Sadeh et al., 1989, Sadeh et al., 1995) in the natural home environment. Actigraphy has been validated in comparison with polysomnography showing agreement rates in healthy individuals of up to 90 % (Sadeh et al., 1995, Ancoli-Israel et al., 2003, Acebo and LeBourgeois, 2006). The method is less reliable in populations suffering from sleep disorders (Sadeh and Acebo, 2002, Ancoli-Israel et al., 2003, Sivertsen et al., 2006).

Individuals were instructed to wear the actigraph on their non-dominant wrist and to maintain their normal sleep habits. Subjects also completed a sleep diary, in which they were requested to note bedtime, time of lights off, sleep latency, wake time, get up time and wake phases during the night. Additionally, they had to write down the amount of alcohol and caffeine consumption, periods where they did not wear the monitor during the day (i.e. due to sport activities), any illness, and smokers had to note the number of cigarettes they were smoking during the day. Actigraphy data were stored in two-minute epochs and subsequent estimation of habitual sleep duration was based on the time spent in bed, including any wakefulness within this period. Recorded data were analysed by a sleep scoring algorithm developed by Oakley and Ungless of Cambridge Neurotechnology Ltd. (Oakley, 1997). The sum of activity was calculated for each recorded two-minute epoch and compared to a threshold value. Dependent of the value, the epoch was either scored as wakefulness or sleep. Nights, in which the actigraph was off or when subjects reported bad sleep quality in the sleep diary were excluded from the analysis. Six subjects were excluded from the analyses: three because of technical problems with the actimetry, two because of large discrepancies between sleep diary- and actigraphic sleep measures, and one because of antidepressant medication use during the study.

Statistical analysis

To describe the relationships between habitual sleep duration and cognitive performance one-way analyses of covariance (ANCOVA) with the factors ‘group’ (higher-functioning performers/lower-functioning performers) and ‘gender’ as a covariate were performed. The covariate ‘gender’ was taken into account, because we observed significant difference between men and women in habitual sleep duration (two-tailed, unpaired, *t*-test). The

analyses were calculated for sleep duration during work and leisure days, separately. Potential differences in daytime sleepiness and in varying levels of education between higher- and lower- functioning performers were tested by two-tailed, unpaired *t*-tests. Whether cognitive performance in individuals was biased by the distribution of gender or by testing time was controlled for by chi-square tests between higher- and lower-functioning performers. All statistical analyses were performed using SAS 9.1.3 software (SAS Institute, Cary, NC). Significance level was set at $\alpha < 0.05$.

Results

Shorter sleep duration during leisure days is associated with better performance on the Stroop Color-Word task

First, we investigated whether habitual sleep duration differs between higher- and lower-functioning individuals. We found that higher-functioning individuals have shorter habitual sleep, compared to lower-functioning individuals, especially with respect to interference/inhibition control, as measured by the Stroop Color-word task. The difference in habitual sleep duration between higher- and lower-functioning performers on interference/inhibition control equalled more then 30 minutes (Table 2). These findings were based on self-reported and objective sleep duration during leisure days.

Table 2: Shorter habitual sleep duration in higher- than lower-functioning performers on interference/inhibition control

	Habitual sleep duration (h ± SEM)	
Performance level	**Higher**	**Lower**
MCTQ		
n = 220	8.2 ± 0.1	8.8 ± 0.1*
n = 82	8.0 ± 0.2	9.0 ± 0.2*
Actigraphy		
n = 82	7.6 ± 0.1	7.9 ± 0.2*

Mean (± SEM) values of habitual sleep duration during leisure days between higher- (Higher) and lower-(Lower) functioning performers derived from the Munich Chronotype Questionnaire (MCTQ) and from actigraphic measures. Higher-functioning performers need less sleep than lower-functioning performers on interference/inhibition control (Stroop Color-Word Task).
*$p < 0.02$ compared to higher- functioning performers.

On the other hand, attention variability (d2 attention task) and response stereotypy (adjacency in RNG test) were only related to self-reported sleep duration (average sleep duration higher-functioning performers: 8.3 ± 0.2 h, average sleep duration lower-functioning performers: 8.8 ± 0.2 h, $p < 0.05$; two-tailed, unpaired *t*-test). Thus, the differences in sleep duration between higher- and lower- functioning individuals equalled roughly 30 minutes. In contrast, sleep duration, as estimated by actigraphy was not related to attention variability and response stereotypy (data not shown). Furthermore, habitual sleep duration during workdays was not related to interference/inhibition control, attention variability and response stereotypy (data not shown). Furthermore, measures of learning and memory were not related to habitual sleep duration.

Nevertheless, the covariate gender revealed a strong and significant impact on habitual sleep duration in all individuals, indicating different habitual sleep length in women and men (Covariate 'gender': $p < 0.05$). This consistent difference will be discussed later in the result section.

Mixed model ANCOVA revealed for the Stroop Color-Word task similar daytime sleepiness, as measured by the ESS, between higher- (5.8 ± 0.3) and lower- functioning (6.1 ± 0.3) performers ($p > 0.4$, two-tailed, unpaired *t*-test). The level of education was exactly the same in both groups (13.00 ± 0.2 years of education, $p > 0.9$, two-tailed, unpaired, *t*-test). In addition, chi-square analysis revealed uniform distribution of women and men in the group of higher- and lower- functioning performers (chi-square=1.8, $p > 0.1$) and no significant difference between higher- and lower- functioning performers was found for appropriate testing times (chi-square=0.4, $p > 0.5$). Similar results were obtained for all other cognitive test variables (data are not shown).

Distribution of habitual sleep duration in a population of middle-aged, healthy adults

Distributions of self-reported sleep durations on work and leisure days on the MCTQ are shown in Figure 1. On average subjects reported to sleep 1.2 h less during the week (7.3 ± 0.1 h), compared to the weekend (8.5 ± 0.1 h) ($p < 0.001$, two-tailed, paired *t*-test).

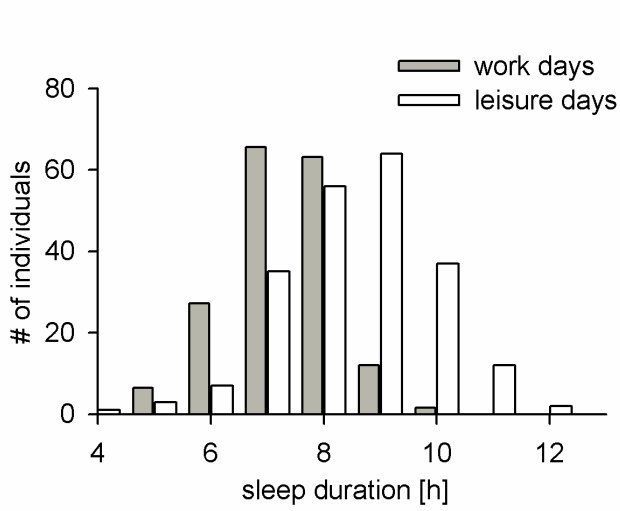

Figure 1: Distribution of habitual sleep duration during work and leisure days
Distribution of self-reported sleep duration on the Munich Chronotype Questionnaire (MCTQ; n=220). Grey bars: workdays. White bars: leisure days.

Gender difference in self-reported sleep duration became apparent, indicating that women sleep on average roughly 0.5 h longer than men ($p < 0.01$, two-tailed, unpaired *t*-test) (Table 3).

Table 3: Habitual sleep duration as measured by questionnaire

MCTQ (h±SEM)	Women (n=105)	Men (n=115)
Workdays:	7.5 ± 0.1**	7.0 ± 0.1**
Leisure days:	8.8 ± 0.1	8.3 ± 0.1

Mean (± SEM) values of self-reported sleep duration as measured by the Munich Chronotype Questionnaire (MCTQ). Women reported to sleep longer than men during work and leisure days ($p < 0.001$, two-tailed, unpaired *t*-test).
**$p < 0.001$ compared to leisure days (two-tailed, paired *t*-test) .

Large variability between self-reported and actigraphy estimated habitual sleep duration in leisure days

Similar results for self-reported (questionnaire) sleep duration during workdays were found in the 35 women and 47 men (24.8 ± 0.4 years) wearing an actigraph as well (Table 4). Nevertheless, when comparing subjective sleep duration with objective estimations of sleep-wake patterns we found a difference in sleep length for leisure days, but not for workdays (Table 4): Individuals overestimated their habitual sleep duration during leisure

days (data derived from the questionnaire) compared to actigraphic estimates of sleep duration. The difference amounted to approximately 51 minutes.

Table 4: Estimated habitual sleep duration

MCTQ (hrs±SEM)	**Women (n=35)**	**Men (n=47)**
Workdays:	7.4 ± 0.2*	7.0 ± 0.2*
Leisure days:	8.9 ± 0.2	8.2 ± 0.2
4-week actigraphy (hrs±SEM)		
Workdays:	7.4 ± 0.1*	7.2 ± 0.1*
Leisure days:	7.8 ± 0.2♦	7.6 ± 0.2♦

The mean number of nights analysed per subject from the actigraphy was 19.1 ± 0.2 nights on workdays and 7.7 ± 0.1 nights on leisure days. Women reported to sleep longer than men during work and leisure days ($p < 0.001$, two-tailed, unpaired *t*-test), which was no longer significant for actigraphy estimated sleep duration ($p > 0.2$, two-tailed, unpaired *t*-test).
*$p < 0.02$ compared to leisure days (two-tailed, paired *t*-test).
♦$p < 0.01$ compared to MCTQ of leisure days (two-tailed, paired *t*-test).

In contrast, there was no difference between self-reported and objective habitual sleep duration during workdays. In addition, correlation analyses between sleep duration derived from questionnaire and actigraphy revealed a significant correlation for sleep duration on workdays ($r = 0.5$, $p < 0.0001$, n=82). This was not the case on leisure days ($r = 0.1$, $p > 0.2$, n=82). In contrast to self-reported sleep duration, actigraphy estimated sleep duration did not differ between women and men ($p > 0.05$, two-tailed, unpaired t-test).

Long sleep duration during the week is associated with long sleep duration during the weekend

To investigate whether an accumulated sleep-debt during the work week may lead to longer sleep duration during the weekend, self-reported (MCTQ, n=220/n=82) and actigraphic (n=82) sleep duration of work and leisure days were correlated (Fig. 2). Correlation analyses described weak, but significant positive association between subjective sleep duration during work and leisure days for all 220 individuals ($r = 0.23$, $p < 0.001$) (Fig. 2A) and also for the subset of 82 individuals, whose actigraphy data were available ($r = 0.25$, $p < 0.03$) (Fig. 2B, black symbols). This finding indicates that longer sleep duration during the week is associated with longer sleep duration during the weekend.

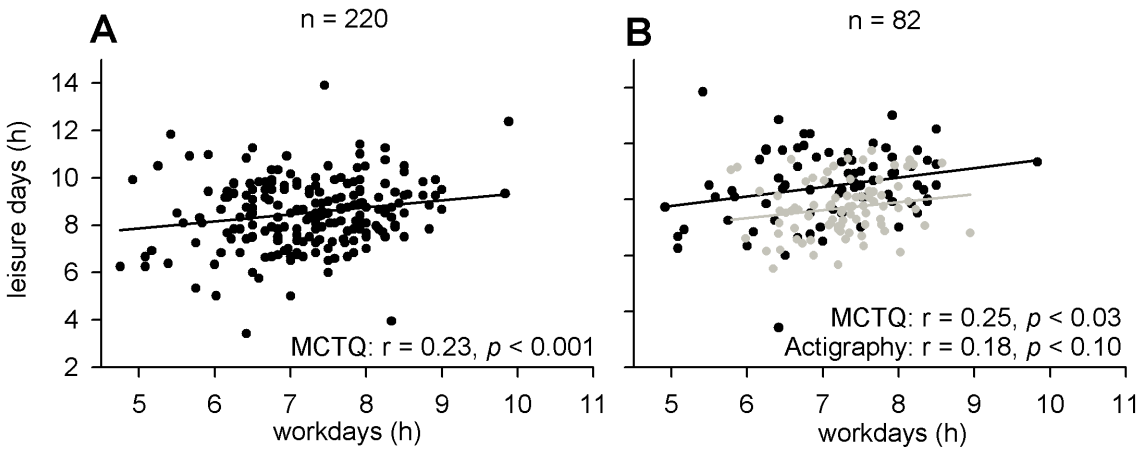

Figure 2: Correlation between habitual sleep duration during work and leisure days
Linear regression line was fitted to the values of self-reported sleep duration during work and leisure days, as estimated by the Munich Chronotype Questionnaire (MCTQ) (black symbols) and as estimated by actigraphy (grey symbols) in 220 subjects (A) and in 82 subjects (B).

In contrast, actigraphic correlation analysis was not significant, but we found a trend pointing in the same direction (Fig. 2B, grey symbols).

Discussion

In this study we examined whether habitual sleep duration differs between healthy, non-sleep-deprived higher- and lower-functioning performers on an executive task. With respect to the available literature in this area, there are several important advantages in the study: Rather than looking at a global measure of "intelligence", done in previous studies (Bodizs et al., 2005, Schabus et al., 2006, Geiger et al., 2010, Gruber et al., 2010), we assessed distinct neuropsychological variables, focusing on executive functions, but also examined attention, learning and memory. In addition, we controlled for the impact of gender, daytime sleepiness and the level of education between higher- and lower- functioning individuals. Habitual sleep duration was estimated based on subjective (questionnaire) and objective (actigraphy and sleep diary) measures. Actigraphy was completed during four consecutive weeks in addition to a sleep diary, which was carefully filled in by all participants.

The principle finding is that self-reported sleep duration on leisure days is inversely associated with performance on a Stroop Color-Word task, which probes the executive function interference/inhibition control. Thus, higher-functioning performers indicate to sleep less than lower-functioning individuals. The association was consistently found and independent of whether habitual sleep duration was assessed by questionnaire or

actigraphy. In addition, attention variability (d2 task) and response stereotypy (adjacency in RNG task) revealed similar findings, but only for self-reported sleep duration. By contrast, measures of learning and memory were not associated with habitual sleep length.

The data presented here suggest that the associations between habitual sleep duration and executive functioning are task specific. Our findings are in line with a recently published paper by Geiger et al. (2010), who found that subjective sleep duration on weekends and fluid IQ were related to each other. In contrast, they showed that full-scale IQ and working memory were not associated to subjective and objective estimates of habitual sleep duration.

To point out, associations between habitual sleep duration and distinct executive functioning were found for sleep duration on leisure days, but not for sleep duration on workdays. We suggest that during workdays sleep may be restricted due to work and other obligations and therefore, habitual sleep duration during leisure days is a more reliable measure of the individual differences in physiological sleep duration. However, by looking at actigraphy data a surprising picture emerged. Habitual sleep duration during leisure days did differ between actigraphic measures and self-reported data, indicating longer subjective (questionnaire) than objective (actigraphy) habitual sleep durations. Evidence from other studies supports the notion that actigraphy data indicate shorter sleep durations than self-reported data (Silva et al., 2007, Lauderdale et al., 2008, Kline et al., 2010), demonstrating that the methodology used, has a large impact on measures of sleep duration (Van Den Berg et al., 2008) and on the interpretation of the results. Differences might result because of imprecise sleep time perception (Watson et al., 2003, Happe et al., 2005).

Nevertheless, actigraphy is an objective estimate of sleep duration, although it has to be considered that the monitor cannot distinguish sleep from inactivity or night-time restlessness during sleep from night-time awakening. Thus, it is important to fill in detailed sleep diaries to improve data validity, as it was done in our study. For the future, we recommend that the relationship between habitual sleep duration and cognitive abilities should not only be obtained by subjective measures (questionnaires), but also include actigraphic measures, which are absolutely essential and should be obtained longer than 7 - 10 days.

On the other hand, the impact of gender on habitual sleep duration is an important issue, which has to be considered when interpreting the results. Consistent with studies reporting

longer sleep duration in women, as estimated from the MCTQ (Roenneberg et al., 2003, Ohayon et al., 2004) we found that women report to sleep roughly 30 minutes more than men. Surprisingly, the effect of gender was no longer present in actigraphic sleep measures. We hypothesize that the sample size of 82 individuals was too small and therefore significant effects were absent due to lack of statistical power. Nevertheless, a study with 1997 Britain's revealed no gender differences in sleep duration as well (Groeger et al., 2004).

In summary, the findings show robust association between distinct measures of executive functions and habitual sleep duration. The difference of habitual sleep duration between higher- and lower-functioning performers in the Stroop Color-Word task is large and robust. Importantly, daytime alertness, education levels and testing times were similar between both groups and as a consequence did not influence our findings. However, cognitive assessment and recordings of actigraphic data were not temporally related. Further research is certainly necessary to better understand the possible functional and complex relationships between habitual sleep duration and cognitive abilities. Importantly, sleep structure and sleep intensity have to be considered as well.

Nevertheless, our findings might have been influenced by genetic factors contributing to these trait-like individual differences in habitual sleep duration between higher- and lower functioning performers (Landolt, 2008a, Crocker and Sehgal, 2010). So far, two genes (hDEC2-P385R and CLOCK) are discussed to contribute to habitual sleep duration in humans (Allebrandt and Roenneberg, 2008, He et al., 2009). Furthermore, animal studies provide evidence for multiple plasticity-related genes involved in cognitive and sleep-wake regulatory processes, as for example BDNF, P-CREB and Arc (Cirelli and Tononi, 2000, Huber et al., 2007). Therefore, we were interested whether the functional p.Val66Met polymorphism of *BDNF* and the p.Asp8Asn polymorphism of *ADA* contribute to sleep-wake regulatory mechanisms in humans (chapter 4 and 5).

Chapter 4

The functional Val66Met polymorphism of *BDNF* state-specifically attenuates EEG delta-waves in nonREM sleep

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Abstract

Animal data suggest that brain-derived neurotrophic factor (BDNF) provides a neurochemical mechanism contributing to the homeostatic facet of sleep regulation. In humans a common functional polymorphism (c.196G>A; NCBI SNP-ID: rs 6265) in the gene encoding BDNF leads to a transition from valine (Val) to methionine (Met) at codon 66 of the protein, which affects activity-dependent secretion of BDNF. Based on the preclinical evidence, we hypothesized that *BDNF* genotype affects waking performance during sleep deprivation and modulates EEG in wakefulness and sleep. Eleven healthy adults, carrying the variant allele (Val/Met) were individually matched to 11 Val/Val homozygotes, to study sleep and the sleep EEG in baseline and recovery nights after 40-hours of prolonged wakefulness. Working memory, subjective sleepiness and well-being, and the waking EEG were quantified at regular intervals throughout sleep deprivation. Consistent with the literature, we found that Val/Met genotype subjects showed reduced response accuracy, a measure for working memory on the 2-back task and reduced deep stage 4 sleep and EEG slow-wave activity (SWA: 0.75-4.5 Hz) in nonREM sleep in both, baseline and recovery nights than Val/Val genotypes. In contrast, subjective sleepiness and well-being were similar in both groups. The genotype-dependent difference in SWA was most pronounced in the first nonREM sleep episode. In addition, compared to Val/Val genotype, attenuated SWA was found in most bipolar EEG derivations along the antero-posterior axis and the build-up of SWA was attenuated in Met allele carriers in the first 30 minutes of the first nonREM sleep episode. Apart from alterations in REM sleep, alpha activity in wakefulness (10 11.5 Hz) was reduced in the former than the latter. Nevertheless, the dynamics and the rebound of the homeostatic process were similar in both genotypes. The data demonstrate robust frequency- and vigilance-state specific effects on the EEG in waking and sleep and suggest that BDNF genotype potently modulates reliable physiological markers of sleep homeostasis in healthy humans.

Introduction

Brain-derived neurotrophic factor (BDNF) is an established mediator of activity dependent synaptic plasticity in the nervous system (Waterhouse and Xu, 2009). In humans, a functional polymorphism of *BDNF* on the chromosome 11q13 produces a valine to methionine amino acid substitution at codon 66 (p.Val66Met) of the 5' pro-*BDNF* sequence. This polymorphism modifies the intracellular distribution and trafficking of the immature pro-*BDNF*, which consequently impairs the activity-dependent secretion and processing of the mature protein (Egan et al., 2003, Lu, 2003). Brain imaging studies in humans revealed that in comparison to individuals homozygous for the Val-allele, healthy Met-allele carriers' hippocampal activation pattern is modulated and their hippocampal N-acetyl aspartate (an intracellular marker of neuronal function) is lower. Moreover, carriers of the variant allele perform worse on tests of memory, on fine motor performance and on executive functions (Egan et al., 2003, Chen et al., 2004).

Sleep is homeostatically regulated (Borbely, 1982), and the level of slow-wave activity (SWA; power density in the 0.75 – 4.5 Hz range) in the electroencephalogram (EEG) in non-rapid-eye-movement (nonREM) sleep is the most reliable physiological marker of sleep homeostasis (Borbély and Achermann, 2005). SWA increases exponentially as a function of the duration of wakefulness and dissipates during sleep (Cajochen et al., 1999, Finelli et al., 2001b, Borbély and Achermann, 2005). Sleep deprivation causes a further increase of SWA and the highest rebound after sleep deprivation is found in the first nonREM sleep episode, when sleep pressure is most pronounced. Independent of sleep pressure, it has been shown that the EEG band between 8 and 16 Hz in nonREM sleep is highly heritable (De Gennaro et al., 2008). Furthermore, the waking and sleep EEG show distinct trait-like, interindividual difference (Buckelmüller et al., 2006, Tinguely et al., 2006), which are larger than the pronounced effects of sleep deprivation (Tucker et al., 2007).

The synaptic homeostasis hypothesis postulates that neuronal plastic changes occurring during wakefulness are causally related to SWA (Tononi and Cirelli, 2003, 2006). BDNF, which has been shown to modulate synaptic changes in the somatosensory cortex of rodents (Genoud et al., 2004) or in hippocampal long-term potentiation (Korte et al., 1995, Tyler et al., 2002) has also been associated with sleep. For example, the expression of *BDNF* in the cerebral cortex of rats is higher during wakefulness compared to sleep (Cirelli and

Tononi, 2000) and the expression of messenger ribonucleic acid (RNA) encoding BDNF was reported to be positively correlated with SWA in recovery sleep (Peyron et al., 1998, Hairston et al., 2004). Moreover, the magnitude of the increase in SWA in subsequent sleep is dependent on the explorative behaviour in rats while being awake, which induces cortical BDNF expression (Huber et al., 2007). Significant positive correlation of intra-cortical injections of either BDNF or a BDNF receptor antagonist in rat cortical brain suggested that BDNF is causally related to sleep homeostasis (Faraguna et al., 2008). In further support of this notion, quantitative trait loci (QTL) analyses in mice showed that the genomic region encoding genes contributing to the signalling cascade of BDNF modify the rate of accumulating sleep pressure during wakefulness (Franken et al., 2001). One distinct QTL including the gene of the neurotrophic receptor, tyrosine kinase B (TrkB), explained almost half of the variance in the rebound in SWA after sleep deprivation. TrkB is the high-affinity receptor for BDNF (Luikart and Parada, 2006).

In this study, we examined in healthy humans whether the functional p.Val66Met polymorphism of *BDNF* contributes to inter-individual differences in neurobehavioral performance, as well as to alterations in the waking and sleep EEG in baseline, during prolonged wakefulness, and in subsequent recovery sleep. Consistent with previous findings, we found that Val/Met allele carriers perform worse than Val/Val allele carriers on a task probing working memory. Apart from other changes in waking and sleep EEG, SWA was specifically reduced in the first nonREM sleep episode in Val/Met compared to Val/Val genotype. This difference was found in baseline and recovery nights, as well as in most bipolar EEG derivation along the antero-posterior axis. Interestingly, Val/Met genotype showed an attenuated build-up of SWA during the first 30 minutes of the first nonREM sleep episode than Val/Val genotype. In contrast, the dynamics and rebound of sleep homeostasis in nonREM sleep were similar between both genotype groups.

Taken together, we provide convergent evidence that genetic variation of BDNF predicts robust inter-individual differences in memory performance and modulates reliable physiological markers of sleep homeostasis in healthy humans.

Materials and Methods

Subject recruitment and genotyping

The local ethics committees for research on human subjects reviewed and approved the study protocol and all experimental procedures. They were conducted according to the principles of the Declaration of Helsinki. All participants provided written informed consent.

Forty-three young adults were genotyped for the p.Val66Met polymorphism of BDNF (c.196A>G; NCBI SNP-ID: rs 6265). Genomic DNA was extracted from 3ml fresh EDTA blood and genotypes were determined with a TaqMan SNP Genotyping Assay (Applied Biosystems, Rotkreuz, Switzerland; Assay ID: C_11592758_10). Allele-specific polymerase chain reaction (PCR) on a MJ Research PTC-225 thermal cycler (MJ Research / Bio-Rad, Reno, NV, USA) was performed. The reaction volume contained 20 ng genomic DNA, 4 μl TaqMan Universal Master Mix (Applied Biosystems, Rotkreuz, Switzerland), 0.4 μl x 20X SNP Genotyping Assay Mix and 1.6 μl H_2O. Annealing temperature was set at 60° C. After running the PCR, an end point fluorescence measurement with the SDS 2.2. Software package (Applied Biosystems, Rotkreuz , Switzerland) was obtained to examine the samples and to discriminate between the specific alleles. All PCRs were independently replicated for data confirmation. The prevalence of the Val/Val genotype was 65.1 % (28/43), of the Met/Met genotype 2.3 % (1/43) and of the Met/Val genotype 32.6 % (14/43). These numbers reflect the expected frequencies occurring in healthy Caucasian populations (Ventriglia et al., 2002, Egan et al., 2003).

Eleven individuals carrying the Val/Met genotype and fulfilling the inclusion criteria (right-handers, non-smokers, between 20-30 years old, body mass index (BMI) between 18.5 and 24.9, no extreme chronotype, Epworth Sleepiness Scale (ESS) score < 10, moderate alcohol and caffeine consumers) were identified. They were closely matched to 11 Val/Val allele carriers with respect to sex, age, BMI, Chronotype ESS, Trait-State Anxiety Inventory (TAI), alcohol and caffeine consumption (Table 1). Women were matched with respect to their phase in the menstrual cycle (follicular phase, luteal phase). All subjects reported to have no medical history of neurological and psychiatric disease and not taking any medication. To exclude poor sleep efficiency and unrecognized sleep disorders, all subjects were polysomnographically screened in the sleep laboratory for one night prior to the study.

Table 1. Subject characteristics

	p.Val66Met BDNF genotype		*p*-value
	Val/Met	**Val/Val**	
Sex (female / male)	4/7	4/7	n/a
Age (years)	24.0 ± 0.8	23.7 ± 0.6	0.3
Alcohol consumption	2.9 ± 0.9	2.9 ± 0.6	0.9
Caffeine consumption	116.4 ± 30.2	124.1 ± 27.1	0.8
Body mass index (kg/m2)	22.2 ± 0.4	21.6 ± 0.4	0.3
Trait Anxiety	36.8 ± 2.4	38.7 ± 2.2	0.4
Diurnal preference	3.2 ± 0.1	2.9 ± 0.3	0.3
Daytime sleepiness	7.5 ± 0.6	6.6 ± 0.5	0.3

Values represent means ± SEM (n = 11 per group).
Values of caffeine consumption were based on the following average caffeine content per serving: Coffee: 100 mg; Ceylon or green tea: 30 mg; Cola drink: 40 mg (2 dl); Energy drink: 80 mg (2 dl); Chocolate: 50 mg (100 g). Trait anxiety, diurnal preference ("Chronotype"), and daytime sleepiness were assessed with the Trait-State Anxiety Inventory (Spielberger et al., 1970), the Horne-Östberg Morningness-Eveningness Questionnaire, and the Epworth Sleepiness Scale (Johns, 1991), respectively. *P*-values refer to two-tailed, paired *t*-tests.

Laboratory-study design

For two weeks prior to admission, study volunteers were asked to abstain from all sources of caffeine (coffee, tea, coke, chocolate, and energy drinks), to wear a wrist activity monitor on the non-dominant arm, and to keep a sleep- wake diary. For 3 days before and during the study, all subjects were instructed to abstain from alcohol and to maintain regular 8-h sleep/16-h wake cycles. Bed times were scheduled from 24:00 to 08:00 h. When subjects did not obey the instructions, they were excluded from the study. After arrival in the sleep laboratory, the breath ethanol concentration was determined and saliva samples for the measurement of caffeine were collected.

All subjects spent 4 nights (time in bed: 24:00 – 08:00) and 2 days in the sleep laboratory. The first night served for adaptation to the laboratory environment, the second night provided the baseline. The following two days and one night subjects were not allowed to sleep, thus were kept awake for 40 hours (constant supervision by members of the research team, constant temperature, light intensity < 150 lux). The last night of the study served as a recovery night from prolonged wakefulness.

Performance on working memory and subjective state

Working memory was assessed by a verbal 2-back task (Kirchner, 1958) administered at 6-hours intervals, starting at 8.45 o'clock on day one of prolonged wakefulness. Subjects were asked to compare a single consonant, presented on a computer screen with a consonant presented 2 trials earlier. When the target letter was the same letter as two trials before (e.g. f-M-F), subjects responded with their right index finger. By contrast, when the two consonants were different, they had to respond with their right middle finger. Individuals were instructed to respond as fast as possible and as accurately as possible. The task consisted of 24 targets and 56 non-targets, which were presented in random intervals. Each session was preceded by a short practice block without feedback including three targets and seven non-targets. A training session on the evening before study start was conducted in all subjects. The task lasted roughly 10 minutes. Response accuracy (percentage of correct responses) and response speed (reciprocal values of reaction time) were calculated for every individual and session. The first session after awakening from baseline sleep was excluded from analyses due to sleep inertia.

To measure subjective sleepiness, participants filled in a German translation of the Stanford Sleepiness Scale (SSS) (Sturm and Clarenbach, 1997). The questionnaire was administered during continuous wakefulness at 3-hour intervals, starting at 8:15 on day one. Additionally, subjective well-being was assessed by von Zerssens's "Befindlichkeits-Skala (Bf-S)" (von Zerssen et al., 1987), which was administered at 10:45 and 22:45 hours on day 1 (baseline) and 2 (sleep deprivation).

Waking EEG recordings

The waking EEG, the mental electromyogram (EMG), the bipolar electrooculogram (EOG) and the electrocardiogram (ECG) were systematically assessed in 14 sessions at 3-hour intervals starting 15 minutes after lights-on from the baseline night. To record the waking EEG, participants had to relax comfortably on a chair and to place their chin on an individually adjusted head-rest in the sleeping room. A 3-minute period with eyes closed was followed by a 5-min period with eyes open while subjects were fixating a black dot at a distance of 3 m attached to the wall. When individuals tended to fall asleep (i.e. reduced EEG alpha activity; rolling eye movements), they were alerted by addressing them over the

intercom. One hour before each waking EEG recording, subjects had to stay in the laboratory and 15 minutes before all recordings they were by themselves in their bedroom.
All bioelectric signals were recorded with Rembrandt Datalab® (Version 8; Embla Systems, Broomfield, CO, USA) and the polygraphic amplifier Artisan® (Micromed, Mogliano Veneto, Italy). Analog signals were conditioned by a high-pass filter (EEG: -3 dB at 0.15 Hz; EMG: 10 Hz; ECG: 1 Hz) and an antialiasing low-pass filter (-3 dB at 67.2 Hz), digitized and transmitted via fiber-optic cables to a personal computer. Data were sampled with a frequency of 256 Hz. The EEG signal was recorded from 1 referential (C3A2) and 8 bipolar derivations (frontocentral derivation (FC), centroparietal derivation (CP), parieto-occipital derivation (PO)) along the left and right anterior-posterior axes. Artifacts in all derivations were visually identified. The power spectra (Fast Fourier Transform, Hanning window) of artifact-free, 50 %-overlapping 2-s epochs were computed with MATLAB® (The MathWorks Inc, Natick, MA, USA). The absolute and relative (recording sessions at 8:00, 11:00, 14:00, 17:00 and 20:00 on day 1 of baseline were divided by the corresponding values on day 2 of sleep deprivation) EEG power spectra between 0 and 20 Hz (0.5-Hz resolution) of the 5-min periods with eyes open of the derivation C3A2 were computed und are reported.

Sleep EEG recordings

During all experimental nights continuous recordings of EEG, EOG, EMG, and ECG were performed in the same way as during wakefulness. Recorded data were visually scored with Rembrandt Analysis Manager® (Version 8; Embla Systems, Broomfield, CO, USA) for 20-s epochs (C3A2 derivation) to asign standard sleep stages (Rechtschaffen and Kales, 1968). Four-s EEG spectra (FFT routine, Hanning window, 0.25-Hz resolution) of all derivations were calculated with MATLAB® (The MathWorks Inc, Natick, MA, USA), averaged over consecutive 5 epochs, and matched with the sleep scores. Twenty-second epochs with movement- and arousal related artifacts were visually identified and eliminated. To compute all-night power spectra (0.25-Hz resolution) in non-REM sleep (stages 2, 3 and 4) and REM sleep, all artifact-free 20-s values were averaged. In recovery nights, data analysis was restricted to the first 8-hours of the 10-hour sleep opportunities. Absolute and relative (either to baseline night for nonREM and REM sleep or to cycle one of nonREM sleep) EEG power between 0 and 20 Hz was computed for consecutive 0.25-Hz. In addition, SWA (0.75 - 4.5 Hz) in nonREM (stages 2, 3 and 4) sleep was computed. Analyses were performed for the

C3A2 derivation and for all 8 bipolar derivations (FC, CP, PO) along the left and right anterior-posterior axes. Due to similar SWA in left and right hemispheres mean values over left and right FC, CP and PO derivations are reported.

Data analysis and statistics

Working memory, subjective sleepiness, well-being, sleep architecture, sleep and waking EEG in baseline, during prolonged wakefulness and in recovery were analyzed in Val/Met and Val/Val genotype subjects. All statistical analyses were performed with SAS 9.1.3 software (SAS Institute, Cary, NC). If variables were not normally distributed before statistical testing, they were either log-transformed (absolute data of EEG) or analyzed by non-parametric tests. Two- and 3-way mixed model analyses of variance (ANOVA) with the within-subjects factors 'genotype' (Val/Met, Val/Val), 'session' (6 sessions of 2-back task and SSS, 4 sessions of Bf-S), 'condition' (baseline, recovery/deprivation), 'nonREM sleep episode' (episodes 1-4) and derivation (FC, CP, PO), as well as their interactions were performed. If appropriate, two-tailed, paired t-tests or Tukey's test were performed. The significance level for statistical tests was set at $\alpha < 0.05$. If statistical results are not reported in the text, they are found in the legend of the respective figure.

Results

Genotype dependent difference in working memory independent of sleep pressure

Working memory was measured on a verbal 2-back task. Consistent with previous studies (Egan et al., 2003, Hariri et al., 2003), we found that Val/Met allele carriers exhibited impaired response accuracy when compared to Val/Val allele carriers (Fig. 1A). Irrespective of genotype, response accuracy showed a diurnal modulation and was reduced by roughly 10 % after sleep deprivation. The difference between the genotypes was not modulated by sleep deprivation. In contrast to accuracy, response speed was similar in both genotype groups (data not shown).

To examine whether the difference in working memory was caused by differences between the genotypes in sleepiness and well-being, these variables were quantified at regular time intervals during prolonged waking. Sleep deprivation similarly impaired subjective sleepiness (Fig. 1B) and well-being ('session': $F_{3,48} = 28.9$, $p < 0.001$) in both genotypes. By contrast,

neither subjective sleepiness nor personal well-being were modulated by the p.Val66Met polymorphism of *BDNF*.

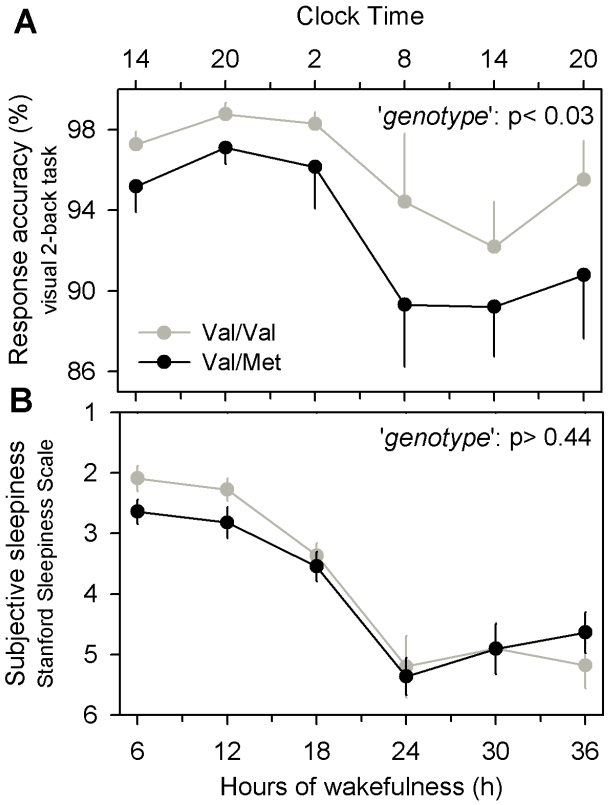

Figure 1. The functional p.Val66Met polymorphism of *BDNF* predicts impaired working memory during prolonged wakefulness independent of sleep pressure and subjective sleepiness

(A) Mean (± SEM) values of the time course of response accuracy (percentage of correct responses), which was measured by the 2-back task, are illustrated. In each subject, six-test sessions, starting at 14:45 in the afternoon at day one were completed. **(B)** Mean (± SEM) scores on the Stanford Sleepiness Scale (SSS) are plotted for consecutive 6-hour intervals, starting at 14:15 of day 1 of prolonged wakefulness.

Black circles: Val/Met genotype (n=11). Grey circles: Val/Val genotype (n=11). Data were analysed with two-way, mixed model ANOVA with the within-subject factors 'genotype' (Val/Met, Val/Val) and 'session' (6 assessments during prolonged waking). Val/Met allele carriers performed worse in response accuracy than Val/Val allele carriers throughout 40 hours of prolonged wakefulness ('genotype': $F_{1,40} = 5.1$, $p < 0.03$), while subjective sleepiness ('genotype': $F_{1,37} = 0.6$, $p > 0.4$) was similar in both groups. Sleep deprivation reduced response accuracy ('session': $F_{5,41} = 11.8$, $p < 0.0001$) and increased subjective sleepiness ('session': $F_{5,68} = 47.9$, $p < 0.0001$) in both groups. The *BDNF* genotypes were not differently affected by sleep deprivation.

Effects of the *BDNF* polymorphism on visually scored sleep variables

Sleep architecture was normal in all individuals, including short sleep latency (< 12 min) and high sleep efficiency (> 93 %). The effects of sleep deprivation on sleep structure were similar in both genotype groups. Compared to baseline, sleep latency, stage 1 sleep, and wakefulness after sleep onset were reduced, whereas slow-wave sleep, total sleep time and sleep efficiency were increased (Table 2). Nevertheless, Val/Met allele carriers spent roughly 20 min less in stage 4 sleep than Val/Val allele carriers in baseline night (Val/Met: 56.6 ± 7.9 min; Val/Val: 73.6 ± 7.9 min), as well as in recovery night (Val/Met: 101.0 ± 11.3 min, Val/Val: 122.0 ± 10.5 min) ('genotype': $F_{1,30} = 5.8$, $p < 0.03$).

Table 2. Visually scored PSG sleep variables during baseline and recovery sleep in Val/Val and Val/Met genotype subjects of *BDNF*

Variable	Val/Met genotype (n=11)		Val/Val genotype (n=11)		'genotype'	'condition'	'genotype x condition'
	Baseline	Recovery	Baseline	Recovery	$F_{1,30}$ (p)	$F_{1,30}$ (p)	$F_{1,30}$ (p)
Episode	467.9 ± 1.8	477.4 ± 0.6	469.8 ± 1.3	476.7 ± 0.8	1.6 (0.21)	68.7 (0.001)	0.4 (0.54)
TST	454.4 ± 2.7	466.7 ± 1.7	448.8 ± 5.1	467.1 ± 1.7	1.1 (0.30)	38.0 (0.001)	1.4 (0.24)
Efficiency	94.7 ± 0.6	97.2 ± 0.4	93.6 ± 1.1	97.3 ± 0.4	1.1 (0.31)	35.6 (0.001)	1.4 (0.24)
SL	9.8 ± 1.3	2.6 ± 0.6	11.6 ± 1.8	3.2 ± 0.8	1.5 (0.24)	62.0 (0.001)	0.4 (0.54)
RL	62.6 ± 3.7	79.5 ± 14.3	68.0 ± 8.5	79.2 ± 12.2	0.8 (0.80)	2.0 (0.17)	0.1 (0.77)
WASO	4.3 ± 1.3	0.7 ± 0.2	10.2 ± 3.7	0.4 ± 0.2	2.1 (0.15)	12.2 (0.02)	2.5 (0.12)
Stage 1	31.2 ± 3.6	13.2 ± 2.0	32.2 ± 4.2	20.3 ± 4.0	3.0 (0.09)	40.9 (0.001)	1.7 (0.20)
Stage 2	220.3 ± 13.2	209.8 ± 12.0	201.8 ± 6.7	189.7 ± 9.8	4.9 0.04)	1.7 (0.21)	0.0 (0.92)
Stage 3	41.2 ± 4.0	46.3 ± 2.9	39.1 ± 4.3	41.8 ± 5.9	0.7 (0.40)	1.0 (0.32)	0.1 (0.76)
Stage 4	56.6 ± 7.9	101.0 ± 11.3	73.6 ± 7.9	122.0 ± 10.5	5.8 (0.02)	34.4 (0.001)	0.1 (0.81)
SWS	97.8 ± 10.5	147.3 ± 10.5	112.8 ± 9.8	163.8 ± 11.2	3.6 (0.07)	36.4 (0.001)	0.0 (0.93)
REM sleep	105.1 ± 5.9	96.4 ± 7.4	102.0 ± 4.4	93.3 ± 8.5	0.4 (0.54)	3.0 (0.10)	0.0 (0.99)
MT	11.2 ± 1.8	10.0 ± 1.4	9.0 ± 1.3	9.2 ± 1.3	2.2 (0.15)	0.2 (0.64)	0.5 (0.49)

Mean values ± SEM in minutes (except sleep efficiency in %) for the first 480 minutes from lights-off. Baseline: baseline night. Recovery: recovery night after 40 hours of wakefulness. Episode: sleep episode, time after sleep onset until final awakening. TST: total sleep time. Efficiency: sleep efficiency, percentage of TST per 480 min. SL: sleep latency, time from lights-off to first occurrence of stage 2. RL: REM sleep latency, time from sleep onset to first occurrence of REM sleep. WASO: waking after sleep onset. SWS: slow-wave sleep. MT: movement time.

F- and p-values: 2 way mixed-model ANOVA with factors 'genotype' (Val/Met, Val/Val), 'condition' (baseline, sleep deprivation) and the interaction 'genotype x condition'.

The p.Val66Met polymorphism of *BDNF* affects the EEG spectra in a state- and frequency-specific manner

To examine whether the p.Val66Met polymorphism of *BDNF* affects the EEG, the spectral composition of the sleep and waking EEG were compared between Val/Met and Val/Val allele carriers. The analyses revealed that the genetic variation of *BDNF* affects the EEG spectrum in a state- and frequency specific manner.

Consistent with reduced homeostatic sleep pressure, EEG power values in nonREM sleep was lower in Val/Met allele carriers than in Val/Val genotype in the frequency bins encompassing the delta and theta, frequency bands (0 - 8.75 Hz) ('genotype': $F_{1,30}$ = 4.8, p < 0.04) (Fig. 2A). The attenuated EEG activity in Val/Met genotype was similar in baseline and recovery nights (no interaction 'genotype x night'), indicating that the genotype-dependent difference is robust against the effect of sleep deprivation. Intriguingly, the genotype-dependent effects on delta/theta frequencies (< 8 Hz) were similar to the difference between baseline and recovery sleep after 40 hours waking in all subjects (Fig. 2D). Additional, genotype-dependent differences in nonREM sleep were present between 10 and 11.5 Hz and 12.5 and 13.5 Hz, whereas the Val/Met genotype exhibited higher activity compared to the Val/Val genotype subjects ('genotype': $F_{1,30}$ = 4.3, p < 0.05), suggesting more superficial sleep.

Analysis of the EEG power spectrum in REM sleep (Fig. 2B) and wakefulness (Fig. 2C) revealed that the *BDNF* genotype affected the EEG in a state and frequency-specific manner. More specifically, in REM sleep Val/Met genotype exhibited attenuated power mainly in the frequency ranges between 6.25 and 16.5 Hz than Val/Val genotype ('genotype': $F_{1,30}$ = 4.4, p < 0.05) (Fig. 2B). In contrast, sleep deprivation enhanced the EEG power spectrum of REM sleep in the delta (1-3 Hz) and reduced EEG power in the alpha frequency bands (9.25 – 9.5 Hz) ('condition': $F_{1,30}$=4.2, p < 0.05). Notably, this reduction in alpha activity after sleep deprivation in subsequent recovery sleep was in contrast to the genotype-dependent changes in the same frequency range (Fig. 2 B/E).

To assess the effects of the *BDNF* genotype and sleep deprivation on the waking EEG, absolute values of the power density spectra for every individual were averaged over the first five test sessions (8:00, 11:00, 14:00, 17:00 and 20:00) of day one (baseline) and day two (sleep deprivation) and compared to each other. As depicted in Figure 2C, *BDNF* genotype

had a strong effect on the EEG power spectrum between 10 and 11.5 Hz, demonstrating attenuated power in Val/Met genotype compared to Val/Val genotype ('genotype': $F_{1,30}$ = 6.2, p < 0.02). On the contrary, sleep deprivation affected power in delta/theta (< 7 Hz) and beta (13 – 16 Hz) frequencies (Fig. 2F).

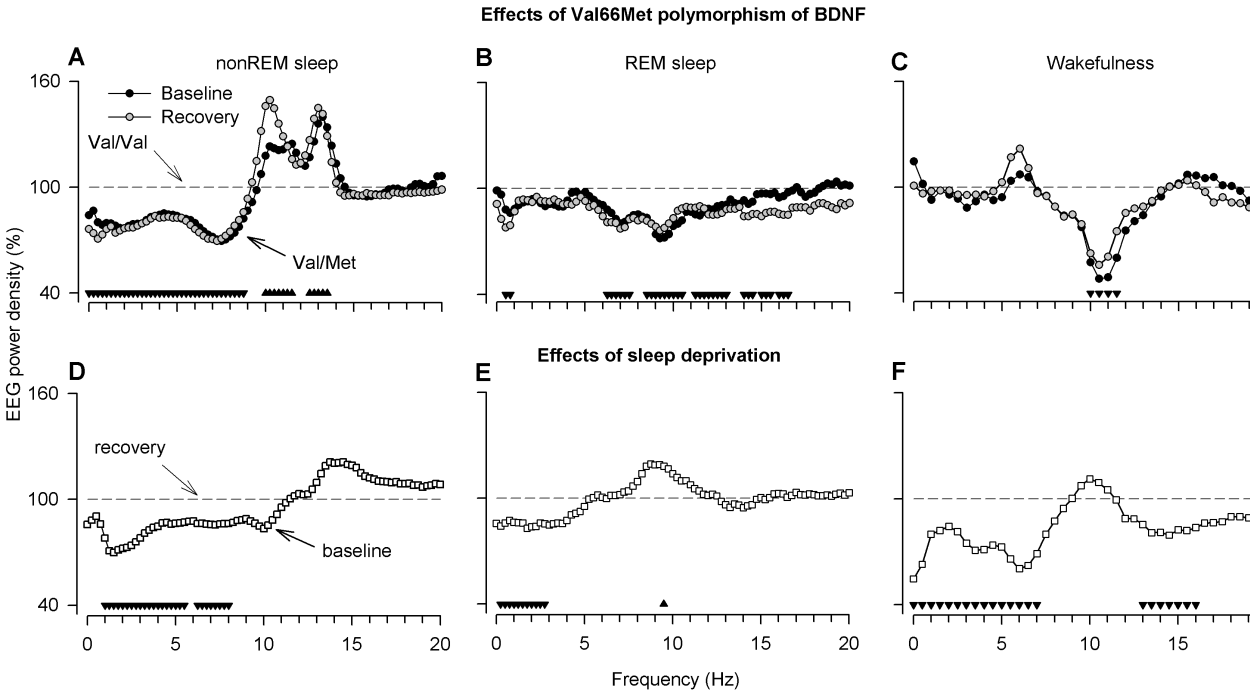

Figure 2. The functional p.Val66Met polymorphism of *BDNF* modulates EEG activity in vigilance/sleep-state specific manner

Relative EEG power densities (C3A2 derivation) in **(A/D)** nonREM sleep (stages 2-4), **(B/E)** REM sleep and **(C/F)** wakefulness (eyes open) are illustrated. **Top panel (A, B, C)**: Absolute power values in individuals with Val/Met genotype (n=11) were expressed as a percentage of the corresponding value of individually-matched Val/Val genotype (horizontal dashed line at 100%). **Bottom panel (D, E, F)**: Absolute power values in baseline were expressed as a percentage of the corresponding values after sleep deprivation (n=22, horizontal dashed line at 100%). The effect of sleep deprivation in wakefulness **(C)** was quantified by dividing the mean values of the waking EEG of the recording sessions at 8:00, 11:00, 14:00, 17:00 and 20:00 on day 1 of baseline by the corresponding values on day 2 of sleep deprivation (horizontal dashed line at 100%). Geometric mean values are plotted for each 0.25-Hz bin in nonREM sleep and REM sleep and for each 0.5-Hz bin in wakefulness. Triangles at the bottom of the respective panels indicate frequency bins for which power significantly differed between Val/Met and Val/Val genotypes (**top panel:** factor: 'genotype': $p < 0.05$) and between baseline and recovery nights (**bottom panel:** factor: 'condition': $p < 0.05$).

Similar to nonREM sleep, the genotype-dependent differences in REM sleep and wakefulness were robust against the effects of sleep deprivation. Nevertheless, the genotype-dependent differences in REM sleep and wakefulness differed from those of sleep deprivation.

In summary, the data show that the p.Val66Met polymorphism of *BDNF* predicts inter-individual differences in the EEG in a state-specific manner. The effects on the all-night

power spectrum in nonREM sleep may be reminiscent of the effects of sleep deprivation and were, thus, analyzed in more detail.

Evolution of EEG power spectra over consecutive nonREM sleep episodes in baseline and recovery nights

To quantify the evolution of EEG power values across the night, absolute EEG activity in nonREM sleep in episodes 2 to 4 was expressed relative to the corresponding values in the first nonREM sleep episode (Fig. 3). Three-way ANOVA analyses showed that the *BDNF* genotype modulated EEG power in the delta, theta, alpha and sigma frequency ranges across the nonREM sleep episodes in both baseline and recovery nights ('genotype': $F_{1,30}$ = 3.9, $p < 0.05$). The progressive decline across the first four consecutive nonREM sleep episodes was similar in both genotype groups (no interaction genotype x nonREM sleep episode) and sleep deprivation did not modulate the effects of the *BDNF* genotype in subsequent sleep (no interaction 'genotype x night'). More specifically, independent of the *BDNF* genotype, EEG power in the delta, theta and alpha band decreased and EEG power in the sigma/beta range increased significantly across all nonREM sleep episodes in both, baseline and recovery nights ('nonREM sleep episodes': $F_{3,30}$ = 2.8, $p < 0.05$). In a next step, analysis of baseline and recovery night was performed separately. As depicted in Figure 3, two-way ANOVAs confirmed the genotype-dependent differences across all nonREM sleep episodes in baseline and recovery nights.

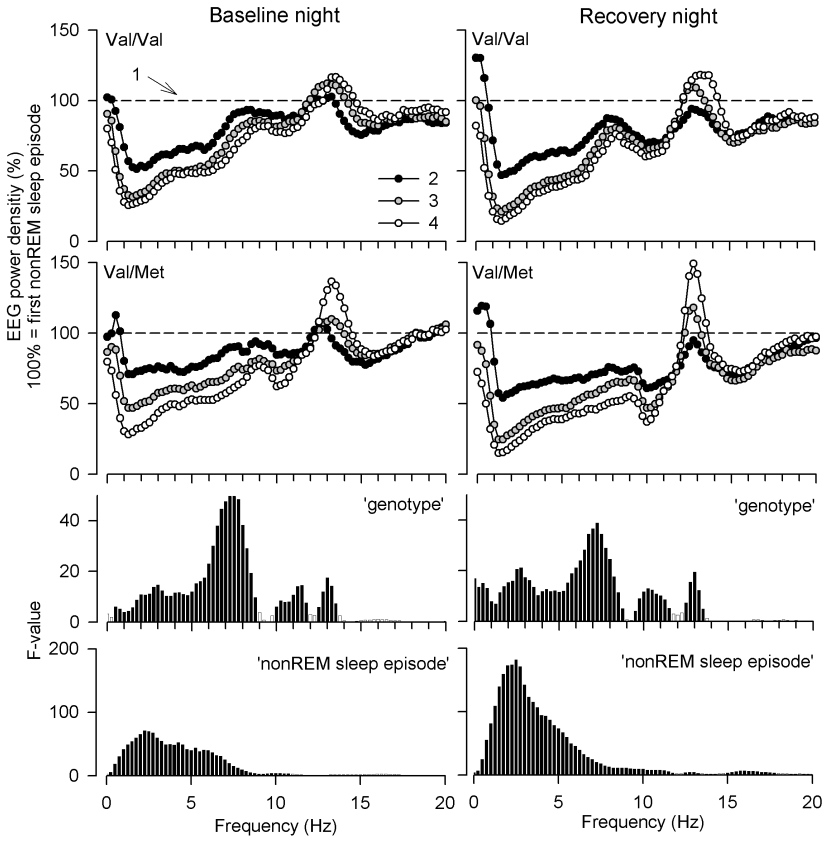

Figure 3. Similar evolution of EEG power spectra in Val/Val genotype (n=11) and Val/Met genotype (n=11) across the first four nonREM sleep episodes in baseline and recovery nights (stages 2-4)

Absolute power values (C3A2 derivation) in each frequency bin in the second (black symbol), third (grey symbol) and fourth (white symbol) nonREM sleep episode were expressed as a percentage of the corresponding absolute values of the first nonREM sleep episode (horizontal dashed line at 100%) (left: baseline; right: recovery). Geometric mean values are plotted for each 0.25-Hz bin. Significant ($p < 0.05$, black bars) and non-significant ($p > 0.05$, white bars) F-values of 'genotype' (Val/Met, Val/Val) and 'nonREM sleep episode' (1-4), which were calculated by two-way, mixed model ANOVA with the within-subject factors 'genotype' and 'nonREMsleep episode' are illustrated on the two bottom panels. No significant interaction 'genotype x nonREM sleep episode' was found for both, baseline and recovery nights.

Dynamics of slow-wave activity (SWA) in nonREM sleep in baseline and recovery nights

To examine the dissipation of sleep pressure in nonREM sleep, non-linear regression analysis of SWA (C3A2 derivation) was computed in Val/Met and Val/Val genotypes in baseline and recovery nights (Fig. 4). The time constant τ of the exponential decline of process S was estimated by the decay of SWA across consecutive nonREM sleep episodes, reflecting the dynamics of the homeostatic process (Rusterholz et al., 2010). As indicated by overlapping 95 % confidence intervals (CI) the time constants did nod differ between Val/Met and Val/Val genotypes in baseline (Val/Met: 261.1 min (95%CI: 133.2 - 388.9); Val/Val: 117.1 min (95%CI: 67.3

- 167.0)) nor in recovery nights (Val/Met: 180.5 min (95%CI: 124.8 – 236.1); Val/Val: 138.9 min (95%CI: 98.1 - 179.6)) (Fig. 4). The goodness of fit was acceptable, as demonstrated by asymptotic R^2 values of > 0.75.

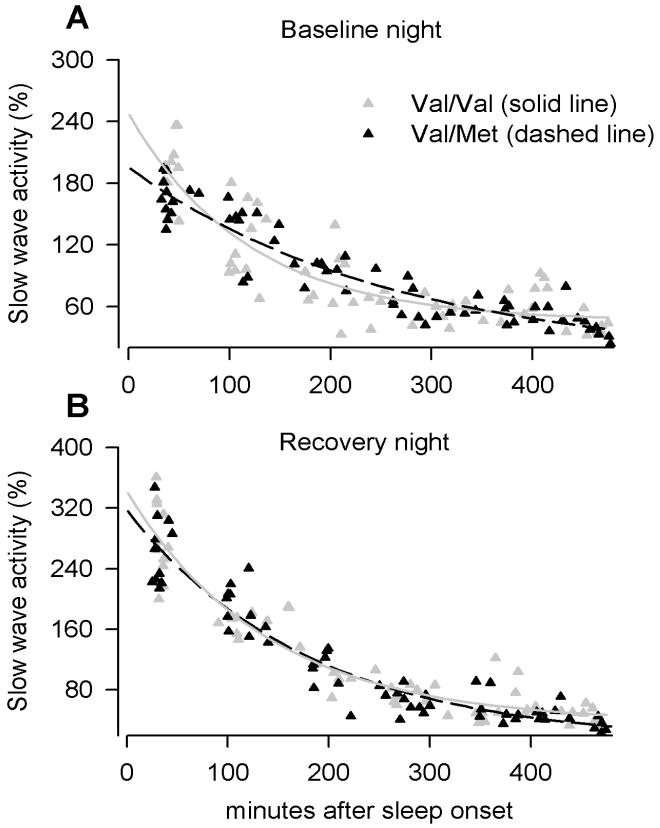

Figure 4. The functional p.Val66Met polymorphism of *BDNF* did not modulate the dynamics of slow-wave activity (SWA) in baseline and recovery night

Exponential decay functions adjusted of relative SWA (0.75 – 4.5 Hz) across nonREM sleep episodes of all-night EEG. Individual SWA values per nonREM sleep episode were plotted at episode midpoint times relative to sleep onset in (**A**) baseline and (**B**) recovery sleep. Lines represent exponential functions for Val/Met allele carriers (black triangles; dashed line) and for Val/Val allele carriers (grey triangles; solid line). Exponential decay fits $SWA_t = SWA_\infty + SWA_0 \times e^{-r*t}$, where t is time, τ the time constant, $(SWA_\infty + SWA_0)$ the initial value and SWA_∞ the lower asymptote. For Val/Met genotype, data were computed on 61 cycles in baseline (goodness of fit coefficient (R^2) = 0.87) and on 62 cycles in recovery sleep (R^2 = 0.76).For Val/Val genotype data were computed on 61 cycles in baseline (R^2 = 0.90) and on 58 cycles in recovery sleep (R^2 = 0.90) . The time constant τ of the exponential decline of process S, which was estimated by the decay of SWA across consecutive nonREM sleep episodes, was similar in both genotype groups in baseline and recovery nights.

Time course of SWA during the first 30 minutes of the first two nonREM sleep episodes

To investigate whether *BDNF* genotype modulated the build-up rate of SWA within the first 30 minutes of nonREM sleep episode one and two, absolute SWA values in the first 30 min of each nonREM sleep episode were calculated for consecutive 2-min epochs (Fig. 5). Val/Met allele carriers show lower power in the first nonREM sleep episode in baseline (Val/Met: 390.3 ± 34.6 μV^2, Val/Val: 596.1 ± 95.4 μV^2) and recovery nights (Val/Met: 745.7 ± 81.8 μV^2, Val/Val: 1040.9 ± 172.0 μV^2) compared to Val/Val allele carriers. In contrast, power in the second nonREM sleep episode was similar in both genotype groups.

In a next step, the rise rate of SWA was computed from the median slopes of these adjacent 2-minute epochs in the first two nonREM sleep episodes in baseline and recovery nights. Independent of genotype and sleep deprivation, the rise rate was steeper in the first, compared to the second nonREM sleep episode ('nonREM sleep episode: $F_{1,70}$ = 13.8, p < 0.001) (Fig. 5).

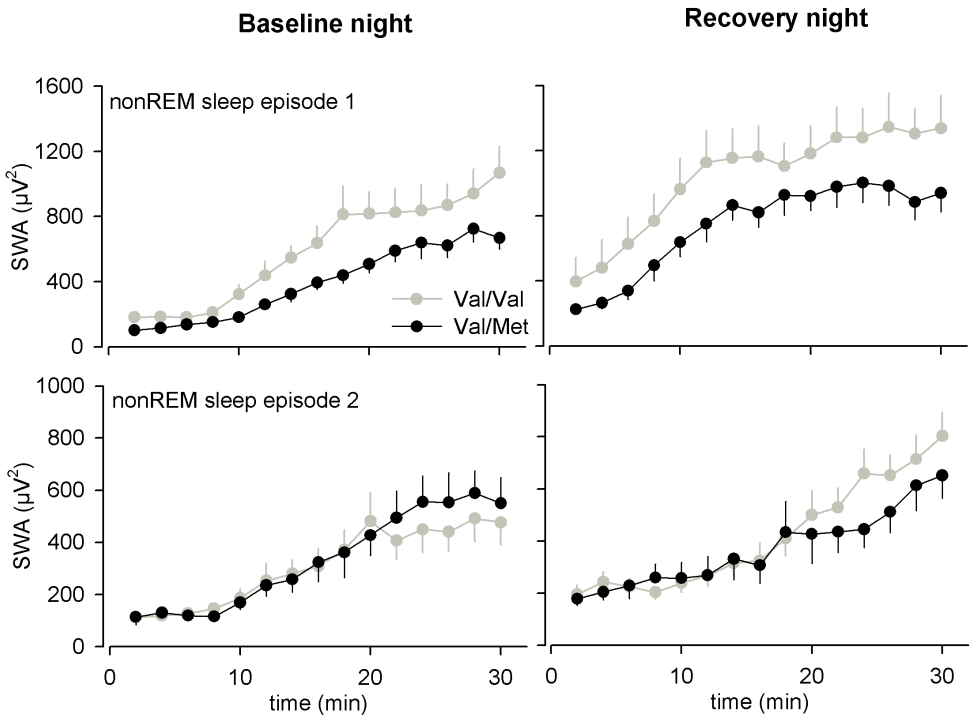

Figure 5. Lower EEG activity and reduced build-up rate of slow-wave activity (SWA) in Val/Met genotype compared to Val/Val genotype subjects in the first nonREM sleep episode
Absolute SWA values (means ± SEM) in the first 30 minutes of each nonREM sleep episode for consecutive 2-min epochs are plotted for Val/Met genotype (black circles) and Val/Val genotype (grey circles) in baseline and recovery nights. Val/Met genotype exhibited attenuated SWA compared to Val/Val genotype in the first nonREM sleep episode in baseline and recovery nights (genotype': $F_{1,30}$= 11.0, p < 0.02). No difference was present in the second nonREM sleep episode ('genotype': $F_{1,30}$= 0.1, p > 0.7) (two-way, mixed model ANOVAs with the within-subject factors 'genotype' (Val/Met, Val/Val) and 'night' (baseline, recovery)). For Val/Met and Val/Val genotype, the rise rates of SWA from the median slopes of adjacent 2-minute epochs in the first 30 minutes for the first and second nonREM sleep episode were calculated for baseline and recovery sleep. Two-way, mixed model ANOVA for the first and second nonREM sleep episode with the within-subject factors 'genotype' (Val/Met, Val/Val) and 'night' (baseline, recovery) revealed that Val/Met genotype showed a reduced build-up rate compared to Val/Val genotype in the first nonREM sleep episode in both, baseline and recovery nights ('genotype': $F_{1,30}$= 7.4, p < 0.02). The difference was no longer present in the second nonREM sleep episode ('genotype': $F_{1,30}$= 0.1, p > 0.7).

The interaction 'genotype x nonREM sleep episode' was significant ('genotype x nonREM sleep episode': $F_{1,70}$ = 5.2, p < 0.03). Post-hoc analyses demonstrated that the difference in the build-up of SWA between the genotypes was restricted to the first nonREM sleep

episode. More specifically, mixed model analyses for each nonREM sleep episode, revealed that the difference was due to an attenuated build-up of SWA in the first nonREM sleep episode in Val/Met genotype than Val/Val genotype in baseline (Val/Met: 27.2 ± 5.4 μV^2/min, Val/Val: 64.3 ± 13.1 μV^2/min) and in recovery nights (Val/Met: 57.0 ± 14.5 μV^2/min, Val/Val: 76.3 ± 14.8 μV^2/min) (Fig. 5). In contrast, the *BDNF* genotype did not modulate the second nonREM sleep episode.

Dissipation of slow-wave activity (SWA) in nonREM sleep across the antero-posterior axis

We were interested whether the genotype-dependent differences in SWA between Val/Met and Val/Val genotype varies with scalp location. Therefore, we analysed SWA in FC, CP and PO bipolar derivations during the first four nonREM sleep episodes (Fig. 6).

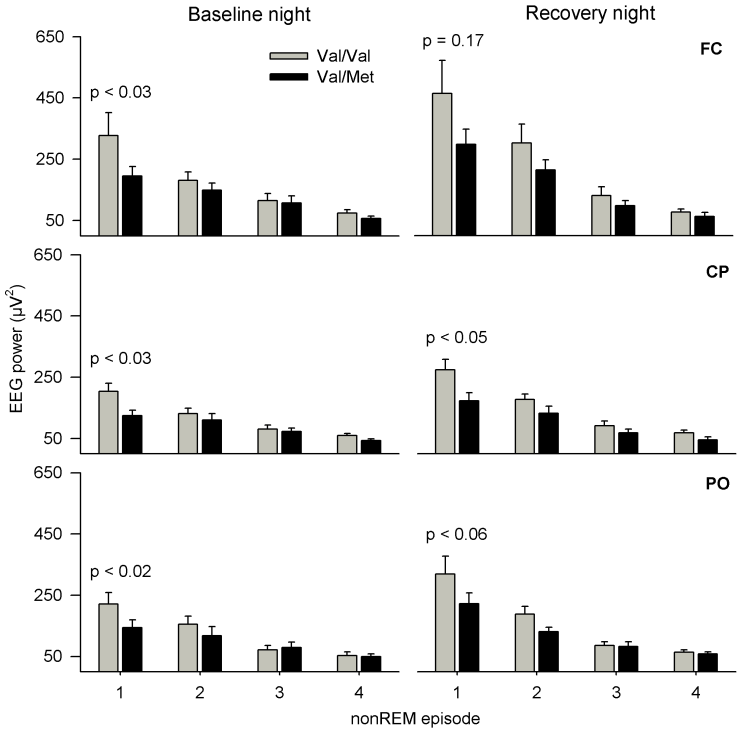

Figure 6. The functional p.Val66Met polymorphism of *BDNF* predicts reduced slow-wave activity (SWA) in the first nonREM sleep episode in both, baseline and recovery nights

Means ± SEM of SWA in frontocentral (FC), centroparietal (CP) and parieto-occipital (PO) derivations are plotted for the first four consecutive nonREM (stages 1-4) sleep episodes in baseline and recovery nights. Black bars: Val/Met allele carriers (n=11), grey bars: Val/Val allele carriers (n=11). Data were analyzed by four-way, mixed model ANOVA with the within-subject factors 'genotype' (Val/Val, Val/Met), 'night' (baseline, recovery), 'nonREM sleep episode' (1-4) and 'location' (FC, CP, PO). Val/Met genotype exhibited attenuated SWA compared to Val/Val genotype ('genotype': $F_{1,122}$= 27.8, $p < 0.001$). Moreover, anterior predominance in SWA was present in baseline and recovery nights ('derivation': $F_{2,377}$= 45.7, $p < 0.001$), whereas SWA decreased across the nights in all derivations ('nonREM sleep episode': $F_{3,257}$ = 403.2, $p < 0.001$; 'night': $F_{1,209}$ = 39.0, $p < 0.001$). No interactions with factor 'genotype' were found. P-values correspond to post-hoc Tukey's tests.

The Val/Met allele carriers exhibited attenuated SWA compared to Val/Val allele carriers. The difference was exclusively present in the first nonREM sleep episode. The regional EEG power distribution was similar in both genotype groups, indicating that the BDNF genotype affected SWA similarly along the anterior-posterior axis. The increase in SWA after sleep deprivation, in subsequent recovery sleep was identical in both genotypes (no interaction genotype x condition). Thus, highest SWA was found in the first nonREM sleep episode and a significant anterior predominance in EEG power was present (Fig. 6), regardless of genotype and night.

Discussion

Here we show for the first time in healthy young adults that the p.Val66Met polymorphism of *BDNF* not only affects memory functions, but also rhythmic brain activity during sleep and wakefulness. The *BDNF* genotype specifically affects delta oscillations in nonREM sleep. Delta oscillations also referred to as SWA reflect sleep need and sleep intensity (Borbély and Achermann, 2005). Therefore, it is remarkably that the genotype-dependent differences in SWA are similar to the effects of sleep deprivation on the EEG spectra, which reflects individuals traits of functional anatomy (Finelli et al., 2001a, Tucker et al., 2007).

Corroborating previous findings that the *BDNF* genotype affects brain functions in healthy humans (Egan et al., 2003, Hariri et al., 2003), we found reduced response accuracy in the Val/Met genotype on a 2-back working memory task, which involves mainly dorsolateral prefrontal and hippocampal areas (Bath and Lee, 2006). The impairments in memory tasks in Met allele carriers compared to homozygous Val allele carriers were associated with decreased synaptic plasticity and modulated activity in the hippocampus (Egan et al., 2003, Hariri et al., 2003). These morphological differences might result from the fact that the p.Val66Met locus in the *BDNF* gene modulates activity-dependent secretion of the peptide (Egan et al., 2003, Binder and Scharfman, 2004). Interestingly, mice, homozygous for the Met allele of BDNF show approximately 30 % reduction in activity-dependent secretion of BDNF from neurons, but the expression of BDNF was similar in Met allele carriers and wild-type controls (Chen et al., 2006). In humans, changes in BDNF levels in blood serum were associated with clinical changes in depression (Brunoni et al., 2008). Interestingly, in this study the treatment of major depression with antidepressants increased BDNF levels. Therefore, we presume that changes in BDNF levels, reflect synaptic changes in the brain,

which as a consequence might results in different memory functions. Unfortunately, we were not able to measure BDNF levels in our individuals. Therefore we can only speculate whether our Met allele carriers exhibited reduced levels of BDNF in the serum compared to the homozygous Val/Val genotype subjects.

Nevertheless, studies in mice, carrying the Met allele exhibited decreased NMDA receptor neurotransmission in the hippocampal CA1 pyramidal neurons compared to wild-type mice (Ninan et al., 2010). Normally, NMDA receptors on the postsynaptic membrane are important for the activation of the calcium-calmodulin kinase II (CAMKII), which regulates early-phase LTP (Fukunaga and Miyamoto, 2000). It is likely that these findings are the reason for impaired working memory performance in Met allele carriers. Intriguingly, worse performance in working memory in Val/Met genotype was robust against the effects of sleep deprivation. Moreover, expected higher subjective sleepiness and worse well-being in Val/Met allele carriers than Val/Val allele carriers were similar in both genotype groups. Therefore, we suggest that the neuronal mechanisms involved in working memory are at least to some extent different to those mechanisms involved in subjective sleepiness.

Furthermore, we found robust frequency- and vigilance-state specific effects on the EEG. Consistent with higher EEG delta power in rat cerebral cortex after BDNF injection (Faraguna et al., 2008), our data show that the functional p.Val66Met polymorphism of *BDNF* modulated the generation of EEG delta/theta waves in nonREM sleep. Individuals with the Met allele exhibited attenuated EEG power in the frequency bands encompassing the delta and theta bands, when compared to Val/Val allele carriers. The difference in the delta/theta power spectra between the *BDNF* genotype was not only present in baseline, but also in recovery night during nonREM sleep. In adolescents, EEG activity in delta, theta, alpha, sigma, and beta range in nonREM sleep was recently associated to cortical gray matter volumes (Buchmann et al., 2010). Notably, the decrease of EEG SWA with age in these healthy adolescences (8-19 years) was similar to the decrease of their grey matter volumes. Together with the findings that Met allele carriers were associated withreduced grey matter volume in the prefrontal cortex (Nemoto et al., 2006) and in parts of the temporal lobes (Montag et al., 2010), our data may suggest that reduced delta activity reflects reduced grey matter volumes in Met allele carriers. This finding of attenuated SWA in Mel allele carriers was further confirmed by the analysis of SWA in the first four nonREM sleep episodes along the anterior-posterior axes. EEG SWA was attenuated in Val/Met

genotype compared to Val/Val genotype in all derivations along the antero-posterior axis and the reduction was exclusively present in the first nonREM sleep episode. Independent of genotype, the decrease of SWA in FC, CP and PO derivations was similar in Val/Met and Val/Val genotype subjects in both, baseline and recovery night and SWA showed the expected anterior predominance as found in previous studies (Finelli et al., 2001b, Borbély and Achermann, 2005). In addition, exponential decay functions adjusted on relative SWA in nonREM sleep for all-night EEG provided evidence that the dynamics of the homeostatic process, as measured by the time constant τ was not affected by the *BDNF* genotype. However, the build-up of SWA during the first 30 minutes of the first nonREM sleep episode was attenuated in Val/Met than Val/Val genotype subjects. Taken together, we show that distinct important physiological markers of the homeostatic regulation of sleep are modulated by the *BDNF* genotype. In contrast, the decline of sleep homeostasis was not affected by the *BDNF* genotype, which indicated that the genetic influences on the sleep EEG are independent of enhanced sleep pressure (De Gennaro et al., 2008).

Not only the sleep EEG, but also the waking EEG were modulated by the p.Val66Met polymorphism of *BDNF*. When compared to Val/Val genotype, the Val/Met genotype exhibited attenuated alpha activity in the waking EEG during prolonged wakefulness. Remarkably, this genotype-dependent difference was in line with findings by Faraguna et al. (2008), who showed a selective increase in the waking EEG power between 11.5 and 12.5 Hz after unilateral injection of BDNF in rat cerebral cortex, when compared to the non-injected, contralateral side. Similar to the effects in nonREM sleep, the differences in our subjects were robust against the effects of sleep deprivation. In further support of this notion, similar trait-like differences were observed by the p.Val158Met polymorphism of *COMT*, when studied in healthy young men (Bodenmann et al., 2009). The polymorphism of *COMT* modulated alpha activity in a similar way, as did the *BDNF* polymorphism (*COMT*: 11 –13 Hz, *BDNF*: 10 – 11.5 Hz).

These data show that polymorphic variations contribute to neurobehavioral performance and sleep-wake regulatory mechanisms. The similar phenotypes for alpha activity may indicate that different single nucleotide polymorphisms similarly affect neuronal networks. So far, few other functional polymorphisms, which have been shown to contribute to waking functions and physiological markers of sleep-wake regulation have been described. The p.Asp8Asn polymorphism of the enzyme adenosine deaminase (*ADA*), which is

responsible for the irreversible breakdown of adenosine to inosine (Fredholm et al., 2005a) contributes to changes in sleep and waking quality in healthy adults and modulates alpha activity in the EEG independent of vigilance state (Rétey et al., 2005, Bachmann et al., in prep.). In addition, genotype-dependent differences in EEG alpha activity (7 – 10 Hz) in nonREM sleep, REM sleep and wakefulness were associated with the 1976T → C polymorphism located in the coding region of the human adenosine A_{2A} receptor gene (*ADORA2A*) (Retey et al., 2005). A variable number tandem repeat polymorphism of *PER3* in humans has also been shown to modulate theta/alpha oscillations in REM sleep and wakefulness, whereas individuals with the 5-repeat allele ($PER^{5/5}$) exhibited higher EEG power compared to 4-repeat allele carriers ($PER^{4/4}$) and appears to modulate the response to sleep deprivation (Viola et al., 2007). In summary, these polymorphic variations, including the distinct genetic variation of *BDNF*, affect several characteristics of waking performance and sleep-wake regulatory mechanisms. To exclude possible interactions of these candidate genes in our study sample, our subjects were genotyped for the *COMT*, *ADA*, *ADORA2A* and *PER3* polymorphisms. Chi-Square analyses in our sample revealed that there were no significant interactions between the particular candidate genes (Table 3).

Table 3. Distribution of Polymorphisms of candidate genes

	COMT			***ADA***		***ADORA2A***			***PER3***		
BDNF	Met/Met	Val/Met	Val/Val	G/A	G/G	C/C	C/T	T/T	4/4	4/5	5/5
Val/Met	3	2	6	2	9	3	5	3	7	4	0
Val/Val	4	2	5	2	9	4	4	3	4	4	3

Chi-Square analysis between individual genetic polymorphisms was performed to control for the distribution of candidates genes being involved in sleep-wake regulation. No significant differences in the distributions were found: $\text{Chi-Square}_{(\text{BDNF vs. COMT})} = 0.2$, $p > 0.8$; $\text{Chi-Square}_{(\text{BDNF vs. ADA})} = 0.0$, $p > 0.9$, $\text{Chi-Square}_{(\text{BDNF vs. ADORA2A})} = 0.3$, $p > 0.8$, $\text{Chi-Square}_{(\text{BDNF vs. PET3})} = 3.8$, $p > 0.1$. BDNF: p.Val66Met brain-derived neurotrophic factor, COMT: p.Val158Met catechol-O-methyltransferase, ADA: Adenosine deaminase, ADORA2A: c.1976T→C polymorphism of the adenosine A_{2A} receptor, PER3: Variable tandem repeat polymorphism of period 3 gene (PER3).

In summary, the functional p.Val66Met polymorphism of *BDNF* activity in healthy adults affects working memory performance, irrespective of subjective sleepiness and well-being. We could show for the first time that BDNF affects nonREM sleep intensity and alpha frequencies in the waking EEG. The attenuated power of SWA in Val/Met genotype was specific for nonREM sleep and stable along the anterior-posterior axes. Moreover the homeostatic build-up during the first 30 minutes of the first nonREM sleep episode was

different between Val/Met and ValVal genotype subjects. However the decline of SWA during the course of the night was similar in both genotype groups. In addition, the effects were similar in baseline and recovery sleep, indicating that the rebound of SWA was not affected by the *BDNF* genotype. The functional relevance of this polymorphism is further supported by the fact that deficits in BDNF production and transportation have also been associated in the pathogenesis of bipolar (Brunoni et al., 2008) and neurodegenerative disorders (Zuccato and Cattaneo, 2009). Several of these pathologies are also associated with impaired cognitive functioning and sleep disturbances.

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Chapter 5

The functional Asp8Asn polymorphism of adenosine deaminase (*ADA*) predicts inter-individual differences in homeostatic sleep pressure

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Manuscript in preparation

Abstract

Supporting an important role for adenosine in sleep-wake regulation, we found previously that the functional c.22G>A (Asp8Asn) polymorphism of adenosine deaminase (*ADA*) predicts baseline differences in slow wave sleep (SWS) and EEG delta activity in nonREM sleep. Here we investigated whether this polymorphism has an impact on waking performance, and electroencephalographic (EEG), behavioral, and biochemical responses to sleep deprivation. Alleles and genotypes of *ADA* were determined in 245 healthy adults (127 male, 118 female; age: 18-40 years). Memory, executive functioning, attention and self-reported habitual sleep duration were systematically quantified in all individuals. To study homeostatic sleep-wake regulation, 11 carriers of the variant allele (Asp/Asn genotype; 5 women, 6 men) were prospectively matched with 11 Asp/Asp homozygotes. Sleep and sleep EEG were recorded in baseline and recovery nights after 40 hours prolonged wakefulness. Waking EEG, psychomotor performance, subjective state, and α-amylase activity in saliva were intermittently quantified throughout sleep deprivation. We found that SWS and low-frequency delta (0.75-1.5 Hz) activity in nonREM sleep in baseline and recovery nights were higher in Asp/Asn genotype than in Asp/Asp genotype. Moreover, EEG theta/alpha (8.5-12 Hz) activity in waking, subjective sleepiness and fatigue, reaction times and lapses on psychomotor vigilance task, as well as salivary α-amylase activity were consistently higher in Asp-allele carriers throughout prolonged wakefulness. By contrast, both genotypes showed similar habitual sleep duration (Munich Chronotype Questionnaire and 4-week wrist-actigraphy) and comparable responses to sleep deprivation. In conclusion, EEG data in sleep and wakefulness, as well as subjective, behavioral, and biochemical markers of vigilance reflecting sleep homeostasis demonstrate that the p.Asp8Asn polymorphism of *ADA* is a key determinant of sleep and waking quality. The findings demonstrate that genetically reduced *ADA* activity is associated with elevated sleep pressure. By contrast, the dynamics of sleep homeostasis and habitual sleep length are not affected.

Introduction

The molecular components and neurochemical bases of sleep homeostasis are poorly understood. The synaptic homeostasis hypothesis of sleep function posits that homeostatically regulated sleep need and sleep intensity are tightly linked to learning processes and the occurrence of synaptic potentiation during wakefulness (Tononi and Cirelli, 2003, 2006). This concept implies that molecular markers of synaptic strength may constitute molecular correlates of sleep homeostasis. Indeed, apart from other molecules such as Homer 1a (Maret et al., 2007) that modulate plastic synaptic processes, the neurotrophin brain-derived neurotrophic factor (BDNF) has recently been suggested to play a causal role in homeostatic regulation of sleep (Huber et al., 2007, Faraguna et al., 2008). In other words, the activity-dependent secretion of BDNF could link synaptic changes during wakefulness with the duration of slow wave sleep (SWS) and EEG slow-wave activity (SWA) in nonREM sleep.

Another system that also plays a well-established role in sleep homeostasis in animals as well as in humans, are the neuromodulator adenosine and its receptors (Basheer et al., 2004, Landolt, 2008b). It may thus be possible that BDNF and adenosine interact, to mediate the consequences of neural activity during wakefulness and sleep. Supporting this notion, *in vitro* data show that the facilitatory action of BDNF on long-term potentiation (LTP) requires endogenous adenosine and adenosine A_{2A} receptor activation (Fontinha et al., 2008). More specifically, enhanced LTP by BDNF is prevented in hippocampal slices when adenosine is removed with adenosine deaminase (ADA) or when adenosine A_{2A} receptors are pharmacologically blocked.

Apart from adenosine kinase (ADK), which phosphorylates adenosine to adenosine-mono-phosphate (AMP), ADA plays an important role in regulating extracellular adenosine levels (Fredholm et al., 2005a). This enzyme is responsible for the irreversible breakdown of adenosine to inosine. Not only ADK (Palchykova et al., 2010) but also ADA may be importantly involved in sleep-wake regulation. Indeed, converging genetic and pharmacological studies in mice and rats indicate an important role for Ada in regulating the rate, at which nonREM sleep need accumulates during wakefulness (Franken et al., 2001), as well as in modulating nonREM sleep intensity (Okada et al., 2003). The latter notion is supported by our own finding in humans that a distinct polymorphic variation of the gene

encoding *ADA* modulates the duration and intensity of SWS in baseline (Rétey et al., 2005). The question, however, whether this single nucleotide polymorphism (SNP) affects sleep homeostasis is unknown.

The human *ADA* gene is located on chromosome 20q13.11 (Tischfield et al., 1974). The most frequent variant allele of *ADA* that is asymptomatic in heterozygous carriers is caused by a Guanine-to-Adenine transition at nucleotide 22 of the *ADA* gene (22G>A; SNP-ID: rs73598374). This polymorphism is translated into an amino acid substitution of asparagine for aspartic acid at codon 8 (p.Asp8Asn) of *ADA* protein (Hirschhorn et al., 1994). Heterozygous carriers of one non-functional allele (Asp/Asn genotype) have 20-30 % lower *ADA* activity in erythrocytes and leucocytes and, therefore, presumably higher levels of endogenous adenosine (Battistuzzi et al., 1981, Riksen et al., 2008).

We investigated the functional consequences of the p.Asp8Asn polymorphism of *ADA* on habitual sleep duration, homeostatic sleep-wake regulation, and waking performance in rested and sleep-deprived state in healthy adults. We collected neurophysiological, behavioral and biochemical data in baseline and after sleep deprivation and predicted based on the previous findings that carriers of the Asp/Asn genotype would habitually sleep longer, show more SWS and SWA, and be more strongly affected by the extension of wakefulness than Asp/Asp homozygotes. To test these hypotheses, we recorded self-reported sleep habits and quantified cognitive performance in 245 healthy volunteers. In as second step, we studied sleep homeostasis in the sleep laboratory in two matched groups of 11 healthy carriers of the Asp/Asn and Asp/Asp genotypes of *ADA*.

Experimental Procedures

Subject recruitment and genotyping

One-hundred-twenty-seven men and 118 women were genotyped. As expected in a healthy Caucasian population (Persico et al., 2000), the prevalence of the Asp/Asn genotype was 12.7 % (31/245), whereas 87.3 % carried the Asp/Asp genotype (214/245). No individual with Asn/Asn genotype was present. Self-reported sleep duration, and attention, memory and executive performance were systematically quantified in all participants (see supporting information, Table S1). Among the carriers of the Asp/Asn genotype, 5 healthy women and 6 healthy men willing to participate in a sleep deprivation study were recruited for the

laboratory experiment. They were prospectively matched with 11 Asp/Asp homozygotes with regard to sex, age, education, habitual alcohol and caffeine intake, body-mass index, trait anxiety, diurnal preference and daytime sleepiness (see supporting information, Table S2). The Cantonal ethics committee for research on human subjects reviewed and approved study protocol and all experimental procedures. They were conducted according to the principles of the Declaration of Helsinki. All participants provided written informed consent.
Genomic DNA was extracted from 3 ml fresh EDTA blood with the Wizard® Genomic DNA purifcation kit (Promega, Dübendorf, Switzerland). Genotypes were determined by allele-specific polymerase chain reaction on a MJ Research PTC-225 thermal cycler (MJ Research / Bio-Rad, Reno, NV, USA). HOT FIREPol® DNA polymerase and the following primers were used: forward primer, 5'-gcg cac gag ggc acc at-3'; reverse primer, 5'-gaa ctc gcc tgs agg agc c-3' (annealing temperature, 67° C). Sequencing was performed by the sanger chain-termination method (Sanger et al., 1977) with an ABI PRISM® 3100 (16 capillaries) genetic analyzer. All analyses were replicated once for independent confirmation of the results.

Habitual sleep duration

Self-reported habitual sleep length on work and leisure days was quantified with the Munich Chronotype Questionnaire (Roenneberg et al., 2003). All participants of the sleep deprivation study wore during 4 weeks in their normal home environment a rest-activity monitor (Actiwatch, Cambridge Neurotechnology Ltd, Cambridge, United Kingdom) on the wrist of their non-dominant arm. Habitual sleep length was estimated form the records of the rest-activity patterns together with inspection of sleep-wake diaries. Note that actigraphy-derived "sleep duration" refers to total time in bed, including possible intrusions of wakefulness.

Attention, memory and executive functioning

Cognitive abilities of all study participants were systematically tested during 2 hours, including attention, learning and memory, and executive functions.
The d2 attention task (Brickenkamp, 1962) is a timed test of selective attention. Fourteen lines of the letters "d" and "p" with 1-4 dashes arranged either individually or in pairs above and below the letters are presented on a sheet of paper. The subjects are allowed 20 s to scan each line, and to mark all letters "d" with 2 dashes. Outcome measures include the total number of processed items, a highly reliable measure of processing speed, the sum of errors

of omission and errors of commission errors, as well as the fluctuation rate across trials (Brickenkamp, 1962).

The validated tests to assess verbal and non-verbal learning efficiency, working memory and executive functions included the Rey Auditory Verbal Learning Test and the Rey Verbal Design Learning Test (Foster et al., 2009), Digit Span Test (Strauss et al., 2006), a Stroop Color-Word Task (Stroop, 1935, Perret, 1974), the Random Number Generation task (Towse, 1998), a Go/No-Go Test (Greenwald et al., 1998), a Design Fluency Test (Regard et al., 1982), and a Letter Fluency Test (Perret, 1974).

Laboratory study to examine homeostatic sleep-wake regulation

Among all genotyped individuals, 22 healthy adults (all Caucasian, mean age: 25.0 ± 0.8 years) were prospectively enrolled into a sleep deprivation study, only based on their p.Asp8Asn genotype of *ADA*. Eleven carriers of the variant allele (Asp/Asn genotype; 5 women, 6 men) and 11 Asp/Asp homozygotes were matched in case-control fashion for sex, age, education level, habitual alcohol and caffeine consumption, body-mass-index, trait anxiety, diurnal preference, and daytime sleepiness (see supporting information, Table S2). Women were matched with respect to the phase in their menstrual cycle (follicular phase, luteal phase). All participants reported to have no history of neurological and psychiatric disease, not taking any medication, and to be moderate alcohol and caffeine consumers. Two pairs of subjects were matched with respect to cigarette smoking (~ 10 cigarettes/day); all other participants were non-smokers.

All subjects were polysomnographically screened in the sleep lab before the study, to exclude poor sleep efficiency and unrecognized, pre-existing sleep disorders.

The experimental protocol consisted of 4 nights and 2 days in the sleep laboratory. The first and second nights (midnight to 8 am) served as adaptation and baseline, respectively. The subsequent 2 days and 1 night subjects were not allowed to sleep, thus, were kept awake for 40 hours. They were constantly supervised by members of the research team. The last night (midnight to 10 am) served as recovery night from sleep deprivation.

Pre-study procedures

For two weeks prior to the study, volunteers were asked to abstain from all sources of caffeine (coffee, tea, coke, chocolate, and energy drinks), to wear a wrist activity monitor on the non-dominant arm, and to keep a sleep-wake diary. For 3 days before and during the

study, all subjects had to also abstain from alcohol, and to maintain regular 8-h sleep:16-h wake cycles. Bed times were scheduled from midnight to 8 am. When not adhering to the directives, subjects were excluded from the study. The smokers were asked to write down the number of cigarettes they smoked per day (not more than ~ 10 cigarettes per day were allowed). During the study, the two pairs of smokers were allowed to smoke at the same pre-defined times, in order to avoid withdrawal.

All-night polysomnography

Polysomnographic recordings including EEG, bipolar electrooculogram (EOG), mental electromyogram (EMG) and electrocardiogram (ECG) were continuously conducted during all experimental nights with Rembrandt Datalab® (Version 8; Embla Systems, Broomfield, CO, USA) and the polygraphic amplifier Artisan® (Micromed, Mogliano Veneto, Italy). Analog signals were conditioned by a high-pass filter (EEG: -3 dB at 0.15 Hz; EMG: 10 Hz; ECG: 1 Hz) and an anti-aliasing low-pass filter (-3 dB at 67.2 Hz), digitized and transmitted via fiber-optic cables to a personal computer. Data were sampled with a frequency of 256 Hz. The EEG was recorded from 1 referential (C3A2) and 8 bipolar derivations along the left and right anteror-posterior axes. The data derived from the C3A2 derivation are reported here.

Sleep stages (Rechtschaffen and Kales, 1968) were visually scored for 20-s epochs with Rembrandt Analysis Manager® (Version 8; Embla Systems, Broomfield, CO, USA). Four-s EEG spectra (FFT routine, Hanning window, 0.25-Hz resolution) were calculated with MATLAB® (The MathWorks Inc, Natick, MA, USA), averaged over consecutive 5 epochs, and matched with the sleep scores. Twenty-second epochs with movement- and arousal related artifacts were visually identified and eliminated. To compute all-night power spectra (0.25 Hz resolution, C3A2 derivation) in nonREM (stages 2, 3 & 4) and REM sleep, all artifact-free 20-s values were averaged. In the recovery nights, data analysis was restricted to the first 8 hours of the 10-hour sleep opportunities.

Waking EEG recordings

During the 40 hours prolonged wakefulness, EEG, EMG, EOC and ECG signals were recorded in 14 sessions at 3-hour intervals in the same way as during the nights, with Rembrandt Datalab® (Version 8; Embla Systems, Broomfield, CO, USA) and polygraphic amplifier Artisan® (Micromed, Mogliano Veneto, Italy). The first recording was scheduled 15 minutes after lights-on from the baseline night. The study participants were instructed to

comfortably relax in a chair, and to place their chin on an individually-adjusted head-rest. A 3-min recording period with eyes closed was followed by a 5-min period with eyes open, while subjects fixated a black dot attached to the wall. At least 1 hour before each waking EEG recording, subjects had to stay in the laboratory (constant temperature, light intensity < 150 lux), and 15 minutes before each recording, they stayed by themselves in their bedroom.

The bioelectric signals were conditioned in the same way as in the polysomnographic recordings. Artifacts in all derivations were visually identified and excluded. The power spectra (Fast Fourier Transform, Hanning window) of artifact-free, 50 %-overlapping, 2-s epochs were computed with MATLAB® (The MathWorks Inc, Natick, MA, USA). The mean power spectra between 0-20 Hz (0.5 Hz resolution, C3A2 derivation) of the 5-min periods with eyes open are reported.

Subjective sleepiness and Profile of Mood States

To quantify the evolution of subjective sleepiness, a validated German version of the Stanford Sleepiness Scale (Sturm and Clarenbach, 1997) was administered 3-hour intervals throughout extended wakefulness (first assessment at 8:10 am after the baseline night). Moreover, subjective sleepiness, vigor, depression and anger were quantified at 4:45 pm on day 1 and day 2 of prolonged wakefulness with the Profile of Mood States (McNair et al., 1971).

Psychomotor vigilance task (PVT)

The psychomotor vigilance task (PVT) (Durmer and Dinges, 2005) is an extensively validated simple reaction time task, which is highly sensitive to inter-individual differences in the impairment of sustained vigilant attention following sleep deprivation (Van Dongen et al., 2003, Schmidt et al., 2007). All participants completed 14 test sessions of 10-min duration at 3-hour intervals during extended wakefulness. The task was implemented on a PC, using the software e-Prime (Psychology Software Tools Inc., Pittsburgh, PA, USA). When a digital millisecond counter started to scroll in the center of the computer screen, subjects had to press a bottom with their right forefinger on a response box connected to the PC. In each session, 100 stimuli were presented (inter-stimulus intervals: 2-10 sec). Subjects received oral instructions and performed one training session on the evening prior to the adaptation night.

Alpha-amylase activity in saliva

Saliva samples (Salivettes®, Sarstedt, Nümbrecht, Germany) were collected at 2-hour intervals throughout prolonged wakefulness, starting at 8 am after the baseline night. Salivary α-amylase (sAA) activity, a recently proposed biomarker of sleep drive, was determined according to previously reported procedures (Nater et al., 2007).

Data analyses and statistics

Cognitive performance, habitual sleep duration, sleep architecture, sleep and waking EEG, subjective sleepiness, mood states, sustained vigilant attention and sAA activity in baseline and after/during sleep deprivation were analyzed in Asp/Asn and Asp/Asp genotype subjects. All statistical analyses were performed with SAS® 9.1.3 software (SAS Institute, Cary, NC). Variables that were not normally distributed (absolute EEG power values and response lapses on the PVT) were transformed to approximate a normal distribution. Two- and 3-way, mixed-model analyses of variance (ANOVA) with the between-subjects factor 'gender' (female, male) and the within-subjects factors 'genotype' (Asp/Asn, Asp/Asp), 'condition' (baseline, recovery/deprivation), 'nonREM sleep episode' (1-4), 'session' (14 assessments during prolonged waking) or 'time' of sAA determination (7 time points) were performed. The significance level was set at $\alpha < 0.05$. If not stated otherwise, only significant effects of factors and interactions are mentioned. Two-tailed, paired and unpaired *t*-tests to localize differences within and between groups were only performed if respective main effects and/or interactions of the ANOVA were significant.

Results

The p.Asp8Asn polymorphism of *ADA* modulates focused attention

Analysis of the d2 attention task in 29 Asp/Asn and 191 Asp/Asp genotype subjects with right-hand dominance revealed that the Asp/Asn genotype processed roughly 30 items less than the Asp/Asp genotype (503 ± 12.6 vs. 534 ± 5.3, $p < 0.04$). This genotype-dependent difference reflects reduced speed in the Asp/Asn genotype, and was also present in the participants of the laboratory experiment (see below). By contrast, the number of commission and omission errors did not differ between the groups (also see supporting information, Table S1). Moreover, performance on various tasks reflecting memory and executive functions was similar in Asp/Asn and Asp/Asp genotypes of *ADA*.

The p.Asp8Asn polymorphism of *ADA* does not affect habitual sleep duration

The Munich Chronotype Questionnaire suggested similar sleep length on work days and leisure days in Asp/Asn (n = 31) and Asp/Asp (n = 214) genotypes (pall > 0.4, data not shown). Four-week rest-activity monitoring in the participants of the laboratory study confirmed this notion. Irrespectively of genotype, habitual sleep duration equalled roughly 7.6-7.7 hours when averaged over work and leisure days (see supporting information, Table S3).

The p.Asp8Asn polymorphism of *ADA* predicts individual differences in slow wave sleep

Both genotype groups showed normal sleep architecture, including short sleep latency and high sleep efficiency in the baseline night (see supporting information, Table S4). Nevertheless, corroborating our previous finding (Rétey et al., 2005), the Asp/Asn genotype subjects spent more time in slow wave sleep than the Asp/Asp genotype subjects (123.9 ± 7.2 vs. 100.3 ± 6.1 min, $p < 0.001$).

Sleep episode duration, total sleep time, sleep efficiency and slow wave sleep increased in the recovery night after sleep loss when compared to the baseline night. On the contrary, sleep latency, wakefulness after sleep onset, stage 1, stage 2, and REM sleep were reduced. These sleep loss-induced changes in sleep architecture were independent of genotype (see supporting information, Table S4).

The p.Asp8Asn polymorphism of *ADA* predicts higher EEG delta activity in nonREM sleep

To characterize possible differences in sleep homeostasis between the genotypes, the EEG oscillations in sleep and wakefulness were quantified with spectral analysis. Low-delta (0.75-1.5 Hz) activity in nonREM sleep was higher in Asp/Asn genotype than in Asp/Asp genotype, both in baseline and recovery nights (Fig. 1A).

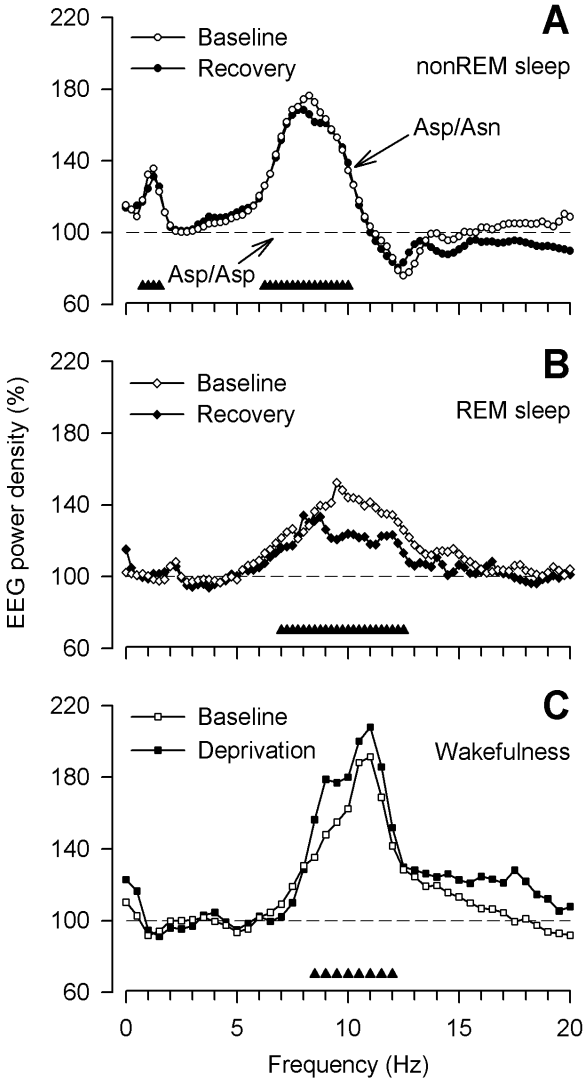

Figure 1. The p.Asp8Asn polymorphism of *ADA* modulates EEG activity in nonREM sleep, REM sleep and wakefulness.
EEG power density (C3A2 derivation) between 0-20 Hz in the Asp/Asn genotype (n = 11) was expressed as a percentage of the corresponding values in the Asp/Asp genotype (n = 11; horizontal dashed line at 100 %). Data in nonREM (A; stages 2, 3, 4) and REM sleep (B) represent all-night values in baseline (white symbols) and recovery nights (black symbols). In the waking EEG (C), averaged power over five 5-min recordings at 8 am, 11 am, 2 pm, 5 pm and 8 pm on day 1 (baseline, white squares) and day 2 (deprivation, black squares) during prolonged wakefulness were analyzed. Geometric means are plotted for each 0.25 Hz bin in nonREM and REM sleep, and for each 0.5 Hz bin in wakefulness. Black triangles denote a significant effect of 'genotype' ($F_{1,30} \geq 4.2$, $p < 0.05$) of a 2-way, mixed-model ANOVA with the within-subject factors 'genotype' (Asp/Asn, Asp/Asp) and 'condition' (baseline, recovery/deprivation). ANOVA revealed no significant 'genotype' x 'condition' interactions.

Low-delta activity is a reliable physiological marker of homeostatically regulated nonREM sleep need, which is highest in the first nonREM sleep episode and declines in the course of the night (Borbély and Achermann, 2005). This time course, as well as the rebound in the first 2 nonREM sleep episodes after prolonged wakefulness, were similar in both genotypes (Fig. 2). The data suggest that the p.Asp8Asn polymorphism of *ADA* does not interfere with the dynamics of sleep homeostasis. Indicated by the generally higher delta activity in nonREM sleep, however, the Asp/Asn genotype may exhibit higher overt sleep pressure than the Asp/Asp genotype. Supporting this hypothesis, the relative rebound in the first nonREM sleep episode was smaller in the former than in the latter (33.3 ± 7.7 vs. 52.8 ± 6.9 %, $p < 0.05$).

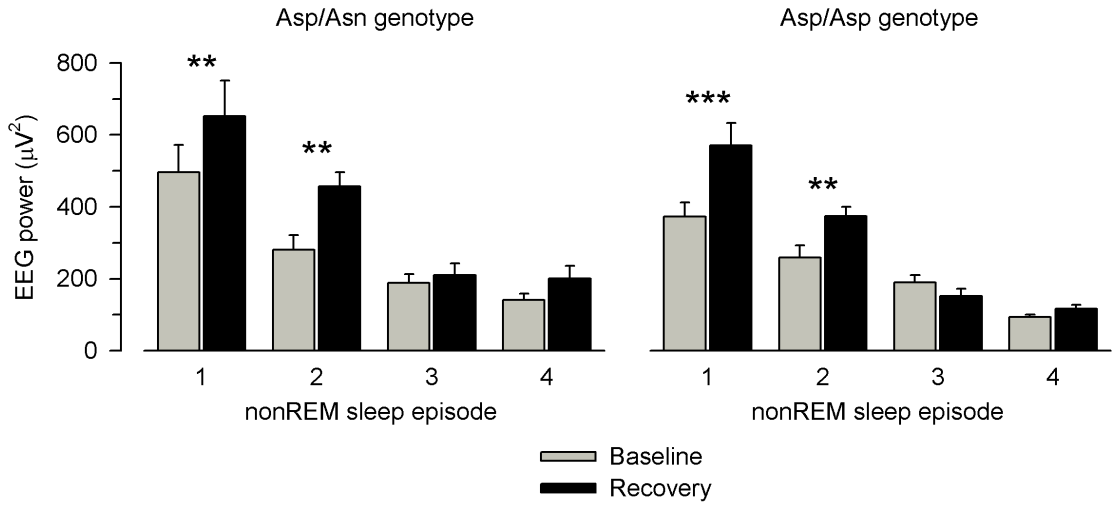

Figure 2. The functional p.Asp8Asp polymorphism of *ADA* does not affect the time course and the sleep loss-induced rebound of EEG low-delta activity (C3A2 derivation, power within 0.75-1.5 Hz) in nonREM sleep (stages 2-4).

Mean delta activity in Asp/Asn (left panel) and Asp/Asp genotypes (right panel) in nonREM sleep episodes 1-4 in baseline (grey bars) and recovery nights (black bars) is plotted. Error bars represent 1 SEM (n = 11). Three-way, mixed-model ANOVA with the within-subject factors 'genotype' (Asp/Asn, Asp/Asp), 'condition' (baseline, recovery) and 'nonREM sleep episode' (1-4) confirmed the significant effect of 'genotype' ('genotype': $F_{1,44}$ = 8.8, p < 0.005), yet revealed no significant 'genotype' x 'condition' and 'genotype' x 'nonREM sleep episode' interactions.

**p < 0.01 (recovery vs. baseline; two-tailed, paired t-test)

***p < 0.001 (recovery vs. baseline; two-tailed, paired t-test)

The p.Asp8Asn polymorphism of *ADA* predicts higher EEG theta/alpha activity in nonREM sleep, REM sleep and wakefulness

The genotype-dependent differences in nonREM sleep were not restricted to the low delta range, but also included theta and alpha oscillations. Irrespective of normal (baseline) or elevated (recovery) sleep pressure, the Asp/Asn genotype subjects exhibited higher activity in the entire 6.25-10 Hz band than the Asp/Asp genotype subjects (Fig. 1A). Suggesting that these differences reflect altered EEG generating mechanisms rather than a genotype-specific difference in sleep-wake regulation, similar changes were also present in REM sleep (Fig. 1B, 7-12.5 Hz), as well as in wakefulness (Fig. 1C, 8.5-12 Hz). To examine whether the p.Asp8Asn polymorphism of *ADA* affects homeostatic and circadian influences on EEG alpha oscillations in waking (Cajochen et al., 2002), the time course of activity in the 8.5-12 Hz range during extended wakefulness was quantified in Asp/Asn and Asn/Asn genotypes. Consistent with the conclusion that this genetic variation does not affect the dynamics of sleep-wake regulation, the genotype-dependent difference in alpha activity persisted throughout sleep deprivation and was not modulated by increasing time awake (Fig. 3A).

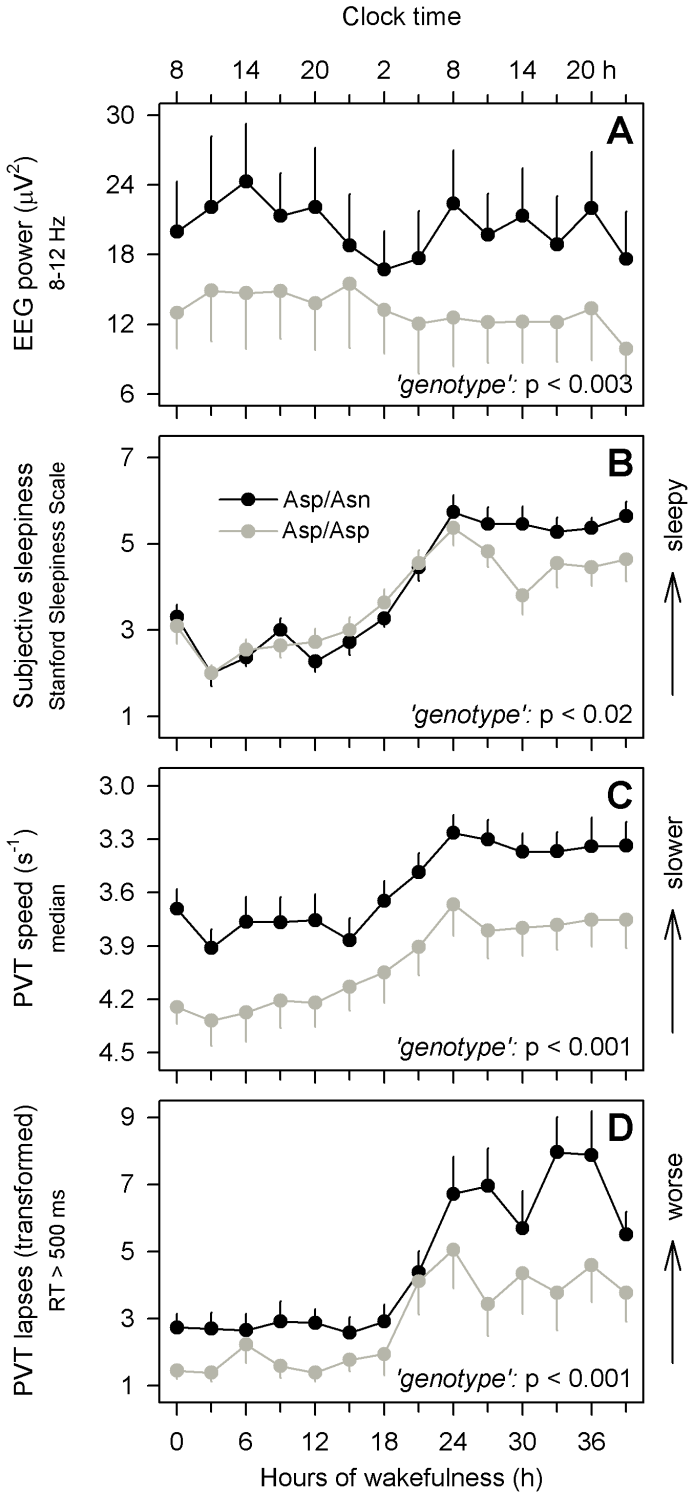

Figure 3. The functional p.Asp8Asn polymorphism of *ADA* predicts higher EEG alpha activity, elevated subjective sleepiness and impaired sustained attention during prolonged wakefulness.

Starting 15 minutes after wakening from the baseline night, 14 test sessions at 3-hour intervals consisting of 5-min waking EEG recording, subjective sleepiness rating and testing of sustained attention were completed in each individual. Ticks on the x-axis are rounded to the nearest hour. Black circles: Asp/Asn genotype (n = 11). Grey circles: Asp/Asp genotype (n = 11). Data were analyzed with 2-way, mixed-model ANOVA with the within-subject factors 'genotype' (Asp/Asn, Asp/Asp) and 'session' (14 assessments during prolonged waking). **(A)** Throughout prolonged wakefulness, EEG activity in the 8.5-12 Hz range was consistently higher in Asp/Asn genotype than in Asp/Asp allele carriers ('genotype': $F_{1,30}$=10.9, $p < 0.003$; 'session': $F_{13,239}$=2.3, $p < 0.007$; 'genotype' x 'session' interaction: $F_{13,159}$=0.2, $p > 0.9$). **(B)** The evolution of subjective sleepiness during sleep deprivation was quantified with a validated German version of the Stanford Sleepiness Scale (Sturm and Clarenbach, 1997). After the night without sleep, sleepiness was higher in the Asp/Asn genotype than in the Asp/Asp genotype ('genotype': $F_{1,78}$= 6.3, $p < 0.02$; 'session': $F_{13,155}$= 42.1, $p < 0.001$; 'genotype' x 'session' interaction: $F_{13,162}$= 1.7, $p < 0.08$). **(C)** & **(D)** Sustained attention during prolonged wakefulness was quantified with the psychomotor vigilance task (PVT) (Durmer and Dinges, 2005).

The time courses of PVT speed (1/median reaction time [RT]) and response lapses (RT > 500 ms, transformed by $\sqrt{x} + \sqrt{(1+x)}$) are illustrated. All RT < 100 ms ("errors of commission") were excluded from analyses. The Asp/Asn genotype performed highly significantly worse that the Asp/Asp genotype (speed: 'genotype': $F_{1,25}$= 15.4, $p < 0.001$; 'session': $F_{13,239}$= 38.6, $p < 0.001$; 'genotype' x 'session' interaction: $F_{13,146}$= 0.3, $p > 0.9$; lapses: 'genotype': $F_{1,66}$= 24.5, $p < 0.001$; 'session': $F_{13,194}$= 19.5, $p < 0.001$; 'genotype' x 'session' interaction: $F_{13,144}$= 1.1, $p > 0.3$).

The p.Asp8Asn polymorphism of *ADA* predicts higher sleepiness during sleep deprivation

Previous work suggested that increased alpha activity in waking EEG with eyes open may be associated with higher subjective sleepiness, and reduced alertness and sustained attention (Oken et al., 2006). Investigating the evolution of subjective sleepiness during sleep deprivation showed that sleepiness increased in both groups with prolonged time awake and was also modulated by circadian influences. The Asp/Asn genotype, however, was more sleepy than the Asp/Asp genotype, particularly after the night without sleep (Fig. 3B). This conclusion was corroborated by the Profile of Mood States (POMS). While sleep loss reduced subjective state in both groups, fatigue was higher and vigor was lower in the Asp/Asn genotype compared to the Asp/Asp genotype (Fig. 4). By contrast, the other POMS subscales were not affected by neither sleep deprivation or genotype (data not shown).

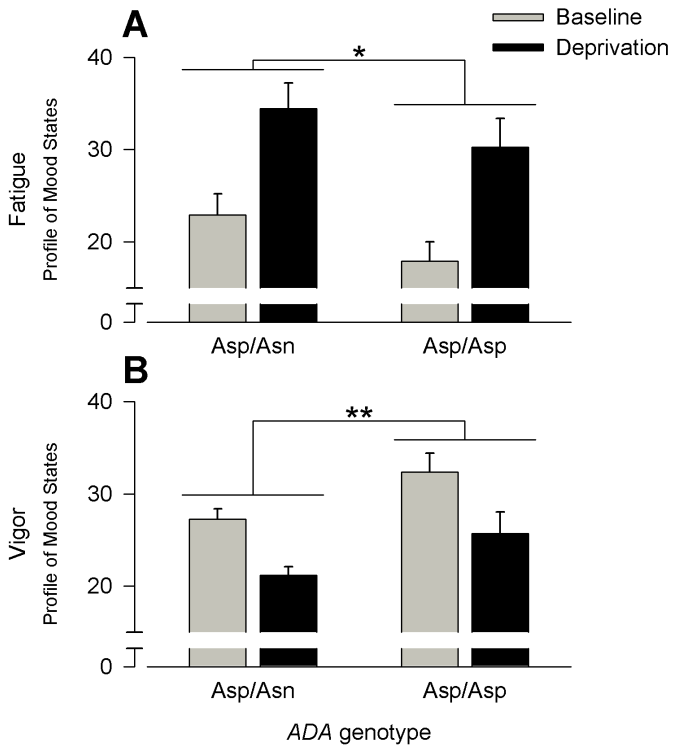

Figure 4. Elevated sleepiness and reduced vigor in Asp/Asn genotype (n = 11) compared to Asp/Asp genotype (n = 11) of *ADA*.
The Profile of Mood States (POMS) was administered at 4:45 pm on days 1 (baseline, grey bars) and 2 (deprivation, black bars) of extended wakefulness. Data represent means + SEM. They were analyzed with 2-way, mixed-model ANOVA with the within-subject factors 'genotype' (Asp/Asn, Asp/Asp) and 'condition' (baseline, deprivation). In rested and sleep-deprived state, fatigue was higher and vigor was lower in the Asp/Asn genotype (fatigue: 'genotype': $F_{1,30}$= 4.2, $p < 0.05$ [*]; 'condition': $F_{1,30}$= 28.8, $p < 0.001$; 'genotype' x 'condition' interaction: $F_{1,30}$= 0.0, $p > 0.8$; vigor: 'genotype': $F_{1,30}$= 9.2, $p < 0.005$ [**]; 'condition': $F_{1,30}$= 16.1, $p < 0.001$; 'genotype' x 'session' interaction: $F_{1,30}$= 0.0, $p > 0.8$).

The p.Asp8Asn polymorphism of *ADA* predicts reduced sustained attention during sleep deprivation

Performance on the psychomotor vigilance task (PVT) is an extensively validated and highly sensitive measure of sustained vigilant attention. Reaction times (RT) and number of response lapses (RT > 500 ms) on the PVT were impaired by sleep loss in both *ADA*

genotypes. However, consistent with increased EEG alpha activity and elevated subjective sleepiness, Asp/Asn genotype subjects performed consistently slower and produced more lapses than Asp/Asp genotype subjects throughout prolonged wakefulness (Figs. 3C & 3D). Importantly, the magnitude of the difference between the genotypes was large, comparable to the effects of one night without sleep. Together, the data confirm that tonic alertness is reduced in healthy individuals with genetically impaired adenosine metabolism.

To further support this conclusion, performance on the d2 attention task was separately examined in the participants of the laboratory experiment. Corroborating the finding in the entire study sample, the Asp/Asn genotype processed significantly fewer items than the Asp/Asp genotype (Fig. 5). The difference reflects reduced speed on the d2 task. Taken together, our findings demonstrate that even under physiological conditions, the functional p.Asp8Asn polymorphism of *ADA* not only modulates sleep structure and intensity, but also importantly contributes to waking quality including sleepiness and attention.

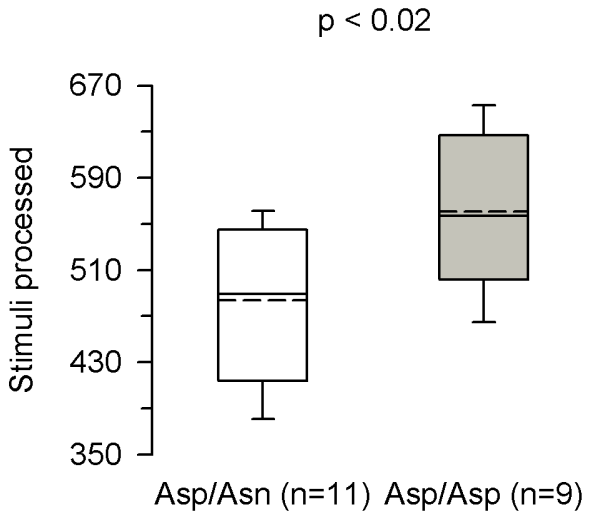

Figure 5. Reduced speed on d2 attention task in the Asp/Asn genotype compared to the Asp/Asp genotype of *ADA*.
The upper and lower lines of the "box and whisker plots" represent the 75th and 25th percentiles of the study sample, whereas the horizontal lines in the middle of the boxes indicate the sample medians (50th percentiles). Mean values of processed items (dashed lines) differed significantly between Asp/Asn (white box) and Asp/Asp (grey box) genotypes (484 ± 19.1 vs. 561 ± 22.6, $p < 0.02$; two-tailed, paired *t*-test). By contrast, the number of errors did not differ between the groups. The data of 2 Asp/Asp genotype subjects participating in the sleep deprivation study were missing.

The p.Asp8Asn polymorphism of *ADA* predicts elevated α-amylase activity in saliva

Salivary α-amylase (sAA) activity, an indirect marker of sympatho-adrenal activity (van Stegeren et al., 2006) was recently proposed to provide a biomarker of sleep drive in flies and humans (Seugnet et al., 2006). We quantified sAA activity throughout prolonged wakefulness and found a pronounced diurnal variation, with highest values in the afternoon and lowest values early at night. In addition, sAA activity in the Asp/Asn genotype was significantly higher than in the Asp/Asp genotype (Fig. 6). These biochemical data are

consistent with our neurophysiological, subjective and behavioral findings, and support the conclusion that the functional Asp/Asn polymorphism of *ADA* is associated with elevated sleep pressure.

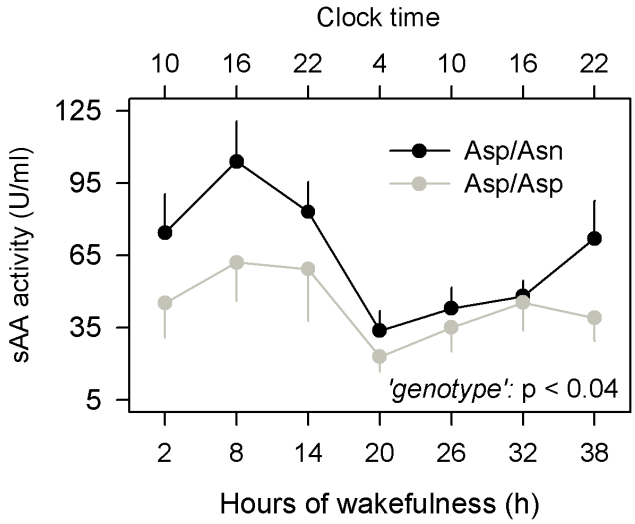

Figure 6. Elevated α-amylase activity in saliva in the Asp/Asn genotype compared to the Asp/Asp genotype of *ADA*. Saliva samples were collected at 2-hour intervals, starting at 8 am on day 1 of prolonged wakefulness.
Salivary α-amylase (sAA) activity (U/ml) was quantified according to previously described procedures (Nater et al., 2007). Values were averaged per 6-hour intervals. Error bars represent SEM (n = 11). The data were analyzed with 2-way, mixed-model ANOVA with the within-subject factors 'genotype' (Asp/Asn, Asp/Asp) and 'time' (7 time points during prolonged waking). ANOVA revealed higher sAA in the Asp/Asn genotype ('genotype': $F_{1,21}$=5.2, $p < 0.04$; 'time': $F_{6,97}$= 12.6, $p < 0.001$; 'genotype' x 'time' interaction: $F_{6,71}$= 0.7, $p > 0.6$).

Discussion

This study demonstrates that healthy adults with genetically reduced ADA activity (Asp/Asn genotype) and presumably higher levels of endogenous adenosine (Riksen et al., 2008) have more SWS and elevated EEG 0.75-1.5 Hz activity in nonREM sleep than individuals with unimpaired ADA activity (Asp/Asp genotype). These differences reflect increased sleep intensity and are invariably present in baseline (also see Rétey et al., 2005), as well as in recovery sleep following sleep deprivation. Moreover, when compared to Asp/Asp genotype subjects, the Asp/Asn genotype subjects exhibit higher theta/alpha (~ 7.5-12 Hz) activity in wakefulness and sleep, are less vigilant (d2 and PVT attention tasks), and show enhanced α-amylase (sAA) activity in saliva. Taken together, these data demonstrate that the functional p.Asp8Asn polymorphism of *ADA* predicts individual differences in overt homeostatic sleep

propensity in healthy individuals, whereas the dynamics of the homeostatic response to sleep deprivation is not affected.

A primary role for adenosine in sleep homeostasis is well established (Basheer et al., 2004, Landolt, 2008b). Recent insights suggest that integrated brain circuits consisting of neurons and astrocytes regulate extracellular adenosine and adenosine-mediated modulation of neural transmission (Haydon and Carmignoto, 2006, Halassa and Haydon, 2010). Thus, astrocytes can be activated by neurotransmitters released from adjacent excitatory synapses and can in turn release chemicals by gliotransmission to act back on these neurons. The primary molecules released by gliotransmission are glutamate, adenosine-tri-phosphate (ATP) and D-serine (Oliet and Mothet, 2006). The ATP is rapidly hydrolyzed to adenosine and modulates synaptic activity by acting on adenosine receptors.

Genetic studies in mice demonstrate the relevance of astrocytic modulation of cortical synapses to the generation of sleep-related EEG rhythms in vivo (Halassa and Haydon, 2010) More specifically, absence of gliotransmission by dominant-negative inhibition of SNARE-dependent membrane fusion (dnSNARE) exclusively in astrocytes decreases the slow (< 1 Hz) oscillation in somatosensory cortex (Fellin et al., 2009). Moreover, dnSNARE mice show reduced delta activity in nonREM sleep in baseline, as well as in recovery sleep after sleep deprivation (Halassa et al., 2009). Reminiscent of the genotype-dependent difference between Asp/Asn and Asp/Asp allele carriers of *ADA*, the difference to wild-type mice is most pronounced in the low-delta band (0.5-1.5 Hz) in nonREM sleep. Remarkably, in contrast to wild-type animals, performance on a novel object recognition task appears unaffected by prolonged wakefulness in dnSNARE mice (Halassa et al., 2009). This phenotype may reflect the difference in performance on d2 and PVT attention tasks between Asp/Asp homozygotes and Asp/Asn allele carriers in humans. Taken together, the data suggest that the overt homeostatic sleep pressure as measured with EEG SWA in nonREM sleep and distinct cognitive consequences of sleep deprivation involve astrocyte-dependent accumulation of adenosine. Because ADA may be more abundantly expressed in astrocytes than in neurons (Fredholm et al., 2005a), our findings could further indicate that the p.Asp8Asn polymorphism of ADA modulates sleep intensity and waking performance by interfering with the astrocyte-dependent regulation of extracellular adenosine.

Adenosine affects sleep and sleep-wake regulation primarily by acting on inhibitory A1 (Bjorness et al., 2009, Halassa et al., 2009) and excitatory A_{2A} receptors (Urade et al., 2003).

A_{2A} receptors are abundantly expressed on γ-amino-butyric-acid (GABA)-ergic neurons of basal ganglia (particularly in putamen and caudate nucleus) and olfactory bulb, and are also present in limbic system and neocortex (Tebano et al., 2005, Bauer and Ishiwata, 2009, Sebastiao and Ribeiro, 2009) Activation of A_{2A} receptors disinhibits sleep-active cells in basal forebrain and ventro-lateral preoptic (VLPO) area of the hypothalamus through pre-synaptic reduction of GABA release, and actively excites distinct VLPO neurons (Chamberlin et al., 2003, Gallopin et al., 2005). These cells play an important role in promoting sleep (McGinty et al., 2004). Mice with A_{2A} receptor loss-of-function have reduced sleep and blunted response to sleep deprivation, as well as to the wake-promoting effects of the adenosine receptor antagonist, caffeine (Urade et al., 2003). The A_{2A} receptor is the main target for caffeine-induced wakefulness (Huang et al., 2005). Consistent with this notion, our own findings in humans demonstrate that the c.1976T>C polymorphism (SNP-ID: rs5751876) of the A_{2A} receptor gene (*ADORA2A*) modulates individual sensitivity to subjective and objective effects of caffeine on sleep (Rétey et al., 2007). Furthermore, caffeine reduces EEG ~ 0.5-2 Hz activity in NREM sleep in rested and sleep deprived state (Landolt et al., 2004, Landolt, 2008b), and differently mitigates the consequences of sleep loss on EEG delta activity and sustained attention depending on *ADORA2A* genotype. Together with the strikingly similar phenotypes on EEG theta/alpha activity in wakefulness and sleep and sustained vigilance attention caused by the c.1976T>C polymorphism of *ADORA2A* (Rétey et al., 2005, Bodenmann, 2009) and the p.Asp8Asn polymorphism of *ADA* (this study), these observations suggest that the repercussions of genetically altered ADA activity are, at least in part, mediated by adenosine A_{2A} receptors.

Stimulation of A_{2A} receptors activates through G_s (or G_{olf} in striatum) proteins the adenylate cyclase (cAMP) / protein kinase A (PKA) pathway (Fredholm et al., 2005b). The PKA stimulates phosphorylation of target proteins through the Thr34 site of DARPP-32 (dopamine- and cAMP-regulated phosphoprotein of relative molecular mass of 32 kDa). Activation of this pathway facilitates the inhibitory function of GABA-ergic, striatopallidal neurons at the cellular and behavioral levels. For example, DARPP-32 is required for the potent motor activation triggered by low doses of caffeine in mice (Lindskog et al., 2002). Genetic studies in humans suggest that this signalling cascade is also involved in mediating the effects of caffeine on sleep. Thus, the DARPP-32 gene lies within a linkage peak on human chromosome 17q that was recently associated in a large Australian twin study with

resistance against the sleep disturbing action of caffeine (Luciano et al., 2007). In view of possible molecular mechanisms underlying sleep homeostasis, it is relevant to note that in hippocampal neurons BDNF-induced LTP, which may be causally related to the homeostatic regulation of sleep (Huber et al., 2007, Faraguna et al., 2008), relies on a cAMP / PKA-dependent mechanism requiring endogenous adenosine and A_{2A} receptor activation (Fontinha et al., 2008).

In conclusion, functional polymorphic variation of *ADA* activity in healthy adults distinctly affects nonREM sleep intensity, theta/alpha frequencies in sleep and waking EEG, subjective sleepiness, attention and α-amylase activity in saliva. These differences do not mirror differences in habitual sleep duration and are robust against the effects of sleep deprivation. Thus, they do not reflect a genotype-dependent alteration in the dynamics of sleep homeostasis. This observation is consistent with recent findings in monozygotic and dizygotic twins, showing that the pronounced genetic influences on the sleep EEG are independent of enhanced sleep pressure (De Gennaro et al., 2008). Moreover, Ada enzymatic activity in rats is not affected by sleep deprivation (Mackiewicz et al., 2003). The data rather suggest an elevated level in overt, homeostatically-regulated sleep propensity in the Asp/Asn genotype compared to Asp/Asp homozygotes, which may be due to elevated in adenosinergic tone at the synapse because of genetically-reduced *ADA* activity. Whether this difference directly underlies the observed phenotypic changes in sleep and wakefulness, or whether it modulates other molecular systems contributing to the homeostatic regulation of sleep propensity, remains to be elucidated.

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Chapter 6

Concluding remarks

In the present thesis, an integrative approach spanning from a molecular level to the system level in humans was used, to investigate whether cognitive abilities and sleep-wake regulation share a common neurobiological basis. We found that distinct genetic variations contributes to inter-individual variability in cognitive functions and physiological markers of sleep homeostasis in healthy humans.

The following steps were taken:

1. Cognitive assessment including executive functioning, attention, learning and memory in 118 women and 125 men
2. Validated questionnaires to assess sleep-wake habits (sleep duration) including diurnal preference (chronotype)
3. Field study of habitual sleep duration, including 4-week wrist actigraphy in 35 women and 47 men
4. Genotyping of the p.Val66Met polymorphism of brain-derived neurotrophic factor (*BDNF*) in 43 participants of previous sleep studies
5. Analysis of distinct variables reflecting sleep homeostasis in 11 Val/Met allele carriers (4 women, 7 men) matched to 11 Val/Val genotype subjects with respect to sex, age and BMI
6. DNA extraction and genotyping of the Aps8Asn polymorphism of adenosine deaminase (*ADA*) in 245 subjects
7. Sleep laboratory study in 11 Asp/Asn genotype subjects (5 women, 6 men) closely matched to 11 Asp/Asp genotype subjects with respect to sex, age and BMI

A synopsis of the major findings is provided in Table 1.

In the following three sections, the main results are put in context with related topics and areas in current research. First, the random number generation (RNG) task is discussed in the context of number processing and executive functioning and its sensitivity to sleep deprivation. Next, the relevance of candidate genes in relation to individual differences in cognitive abilities and sleep-wake regulatory mechanisms are described. Finally, differences

in EEG alpha activity during wakefulness and sleep and their potential role on vigilant attention and working memory are discussed and followed by closing words.

6.1. The RNG task in numerical cognition and its sensitivity to sleep deprivation

The RNG task, which relies on prefrontal function networks demands executive functioning, including the updating and monitoring of generated information. To this end, working memory is required as well as the suppression of habitual counting (Brugger et al., 1996b, Baddeley et al., 1998, Towse, 1998, Towse and Neil, 1998). In addition, RNG bears a spatial component, which has first been shown in healthy individuals, who preferentially selected smaller (1-3) over larger (4-6) numbers (Loetscher and Brugger, 2007). That this preference for selecting small numbers has a spatial basis was suggested by the observation of possible correlations between the magnitude of the preference and various attentional biases towards the left side of space. Consistent with these findings, we found a small number bias (SNB) in our study sample (n=209), which might reflect spatial-attentional interactions in numerical processing (chapter 2). These interactions are suggested to be based on a neural representation of a "mental number line", which extends from left to right in imagined space (Dehaene, 1997). Moreover, we found that prefrontal executive functions are predictive of an individual's orientation bias along the "mental number line". These results indicate that the preference for small numbers is associated with hemispheric differences in specifically prefrontal executive functions. Frontal cortex contributions in number processing have been somehow overlooked, although several studies demonstrate that the involvement of the prefrontal cortex (areas) in number processing is crucial (Jahanshahi et al., 2000, Daniels et al., 2003, Nieder and Dehaene, 2009). Thus, not only the parietal cortex (Hubbard et al., 2005), but also the prefrontal cortex should be considered in future research on numerical cognition. Nevertheless, high inter-individual variability in cognitive processes depends on the influence of various genes (Egan et al., 2003, Savitz et al., 2006, Landolt, 2008, Bodenmann et al., 2009). Recently, it was suggested that genetic variation in the dopaminergic system modulates lateral biases in spatial attention (Greene et al., 2010). More specifically, it has been shown that the genetic variations of two catecholaminergic genes (DBH and DAT) modulate dopamine levels in the cortex and predict differences in

spatial attention. Therefore, we were interested whether the p.Val158Met polymorphism of *COMT* affects SNB in our study sample of 209 subjects. COMT is a major breakdown enzyme of cortical dopamine (Tunbridge et al., 2006). However as depicted in figure 1, the SNB was not modulated by the *COMT* genotype.

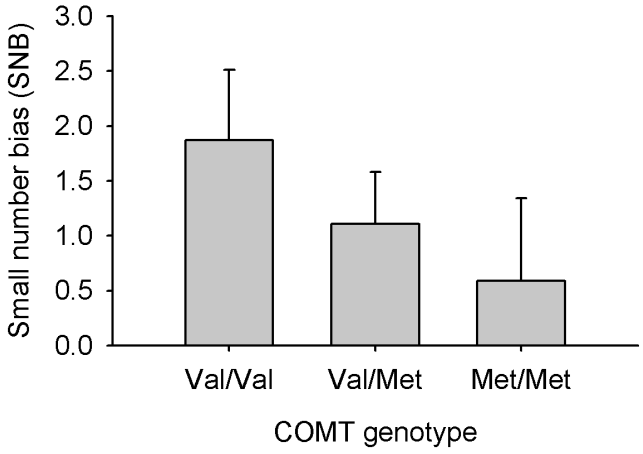

Figure 1: The p.Val158Met polymorphism of *COMT* does not predict a preference for smaller over larger numbers.
Mean values of small number bias (SNB) in Val/Val (n=60), Val/Met (n=88) and Met/Met (n=61) allele carriers of *COMT* genotype. Two-way, mixed-model ANOVA with the between factor 'genotype' revealed no significant difference between the groups ('genotype': $F_{2,205} = 0.9$, $p > 0.3$).

Interestingly, the p.Val158Met polymorphism of *COMT* was found to predict differences in response stereotypy (adjacency on RNG task) (Bodenmann et al., 2009b). Furthermore, response stereotypy was negatively associated with self-reported sleep duration in healthy, not sleep-deprived adults (chapter 3), indicating that better performance in distinct executive functions is associated with subjective sleep duration. Measures of learning and memory were not related to habitual sleep length. These findings are in line with the data of the Zürich longitudinal study, which also showed a negative relationship of similar magnitude between IQ and reported sleep duration in children (Jenni et al., 2009). However, a similar study in children and adolescents, which was recently performed by the same group, confirmed these findings for fluid IQ measures and self-reported, but not for overall IQ and objectively (by actigraphy) estimated sleep duration (Geiger et al., 2010). Taken together, the data show that associations between sleep duration and cognitive abilities are task specific.

The RNG task is also a reliable task to measure the effects of sleep deprivation (Heuer et al., 2005, Gottselig et al., 2006). Sleep deprivation leads to an increase in response stereotypy, to an increase in rule violations and to a decrease in number of responses. Thus, networks of executive functions which rely on the frontal cortex are suffering the most from sleep

deprivation (Durmer and Dinges, 2005). Nevertheless, the sensitivity to sleep deprivation is highly variable among humans. Arguably, genetic influences may be responsible for most of the high inter-individual variability between people (Landolt, 2008a, Tafti, 2009).

Taken together, it is evident that multiple brain areas and overlapping neurotransmitter systems are involved in the different aspects of RNG performance, such as response stereotypy and SNB. The discussed data suggest that dopaminergic mechanisms contribute to some of the different aspects in RNG performance.

In summary, the various aspects of the RNG tasks show that the task is very useful to elucidate inter-individual differences in number processing and executive functioning during baseline and sleep deprived condition. The task is simple to conduct, non-invasive and fast running. Both, young and older people also easily capture its instructions.

6.2. The impact of candidate genes in sleep-wake regulatory mechanisms

Many aspects of sleep show trait-like inter-individual differences in healthy people (Andretic et al., 2008, Landolt, 2008a, Tafti, 2009). It is likely that sleep is not controlled by a single gene locus, but relies on many genes contributing to the variation in sleep characteristics (Franken et al., 2001, Tafti, 2009). One possibility to study the genetic basis of sleep in healthy humans is an approach focusing on candidate genes.

In the present thesis, two candidate genes were studied. Our findings demonstrate that these two functional single nucleotide polymorphisms (SNP) contribute to inter-individual differences in neurobehavioral performance and to the homeostatic sleep-wake regulation. Consistent with previous studies of the functional p.Val66Met polymorphism of *BDNF* on memory functions (Egan et al., 2003), we found that the *BDNF* genotype contributes to working memory performance throughout sleep deprivation. Furthermore, we could show for the first time that it affects physiological markers of sleep homeostasis and modulates the REM sleep and waking EEG in a frequency-specific manner (chapter 4). More specifically, we found that SWA, a reliable physiological marker of nonREM sleep intensity (Borbely, 1982, Borbély and Acherman, 2000) was attenuated along the anterior-posterior axes in Val/Met genotype compared to Val/Val genotype (Fig. 2, left panel). The genotype-dependent differences were similar to the effects of sleep deprivation on the EEG spectra in

subsequent recovery sleep (Tucker et al., 2007, Finelli et al., 2001b). In addition the build-up of SWA during the first 30 minutes of the first nonREM sleep episode was attenuated in Val/Met than Val/Val genotype subjects. Nevertheless, the similar genotype-dependent differences in baseline and recovery sleep indicate the *BDNF* genotype was robust against the effects of sleep deprivation. In addition alpha activity (10 – 11.5 Hz) was attenuated in Val/Met compared to Val/Val genotype subjects. In summary and in accordance with the suggestion that BDNF and sleep homeostasis are causally related (Faraguna et al., 2008), we found that the *BDNF* polymorphism contributes to physiological markers of homeostatic sleep-wake regulation in healthy adults.

In addition, we examined whether the functional p.Asp8Asn polymorphism of *ADA* interferes with sleep homeostasis (chapter 5). Animal and pharmacological studies support the notion that adenosine plays an important role in sleep-wake regulation (Radulovacki et al., 1983, Schwierin et al., 1996, Porkka-Heiskanen et al., 1997, Marks and Birabil, 1998, Okada et al., 2003). In line with previous findings that this polymorphism predicts interindividual differences in high-intensity slow wave sleep in baseline night in healthy young men (Rétey et al., 2005), we found that Asp/Asn genotype subjects spent more time in SWS and exhibited higher sleep intensity in both, baseline and recovery night than Asp/Asp genotypes (Fig. 2, right panel). In addition, Asp/Asn allele carriers show higher alpha activity independent of vigilance-state and their performance on vigilant attention is worse and their subjective sleepiness is higher compared to Asp/Asp allele carriers. In contrast, the dynamics of sleep homeostasis and habitual sleep duration were not affected by the *ADA* genotype. Not only *ADA*, but also the A_{2A} receptors have been shown to be involved in sleep-wake regulatory mechanisms (Urade et al., 2003). In healthy adults, a genetic variation of the A_{2A} receptor, the c.1976T>C polymorphism of the A_{2A} receptor gene (*ADORA2A*) has been shown to affect sleep-wake regulatory mechanisms (Rétey et al., 2005). Very similar to the effects of the *ADA* genotype, the *ADORA2A* polymorphism predicts difference in alpha frequency independent of vigilance state and the same polymorphism differently affects the consequences of sleep loss on EEG delta activity (Bodenmann, 2009).

Other identified candidate genes, which are known to contribute to the variation in sleep characteristics, are represented by the VNTR *PER3* polymorphism. This polymorphism is known to affect not only the circadian system, but also multiple aspects of sleep and wakefulness, as well as the response to sleep loss (Dijk and Archer, 2010). Notably, the *BDNF*,

COMT, ADA and *ADORA2A* polymorphisms modulate not only sleep-wake regulatory mechanisms, but contribute to cognitive processes as well. In addition, they all modulate alpha activity in nonREM sleep, REM sleep and wakefulness in a frequency specific manner. Thus, the functional relevance of these genotype-dependent differences in alpha activity and its possible associations to cognitive processes will be discussed in the next section.

Taken together, our genetic data demonstrate that genotype-dependent differences were stable and reproducible and thus, trait-like. However, it must be considered that the investigation of SNPs does not take into account that the genome might be much more variable. Therefore, the data have to be interpreted with caution and need to be replicated in larger samples (Tabor et al., 2002).

The identification of candidate genes relies on genome-wide associations studies. This technique is also one of the main used techniques for the genetic dissection of normal human sleep. The identification of new candidate genes is a useful approach to further study individual differences in cognitive performance and sleep-wake regulation in healthy humans (Andretic et al., 2008). Therefore, the focus on candidate genes is a useful approach in human sleep research to identify molecular mechanisms underlying the relationships between cognitive performance and sleep-wake regulation and to study inter-individual differences in sleep-wake regulation and neurobehavioral performance. Moreover, the approach advances our understanding of the heritability and biology of many diseases and traits in human subjects (Andretic et al., 2008).

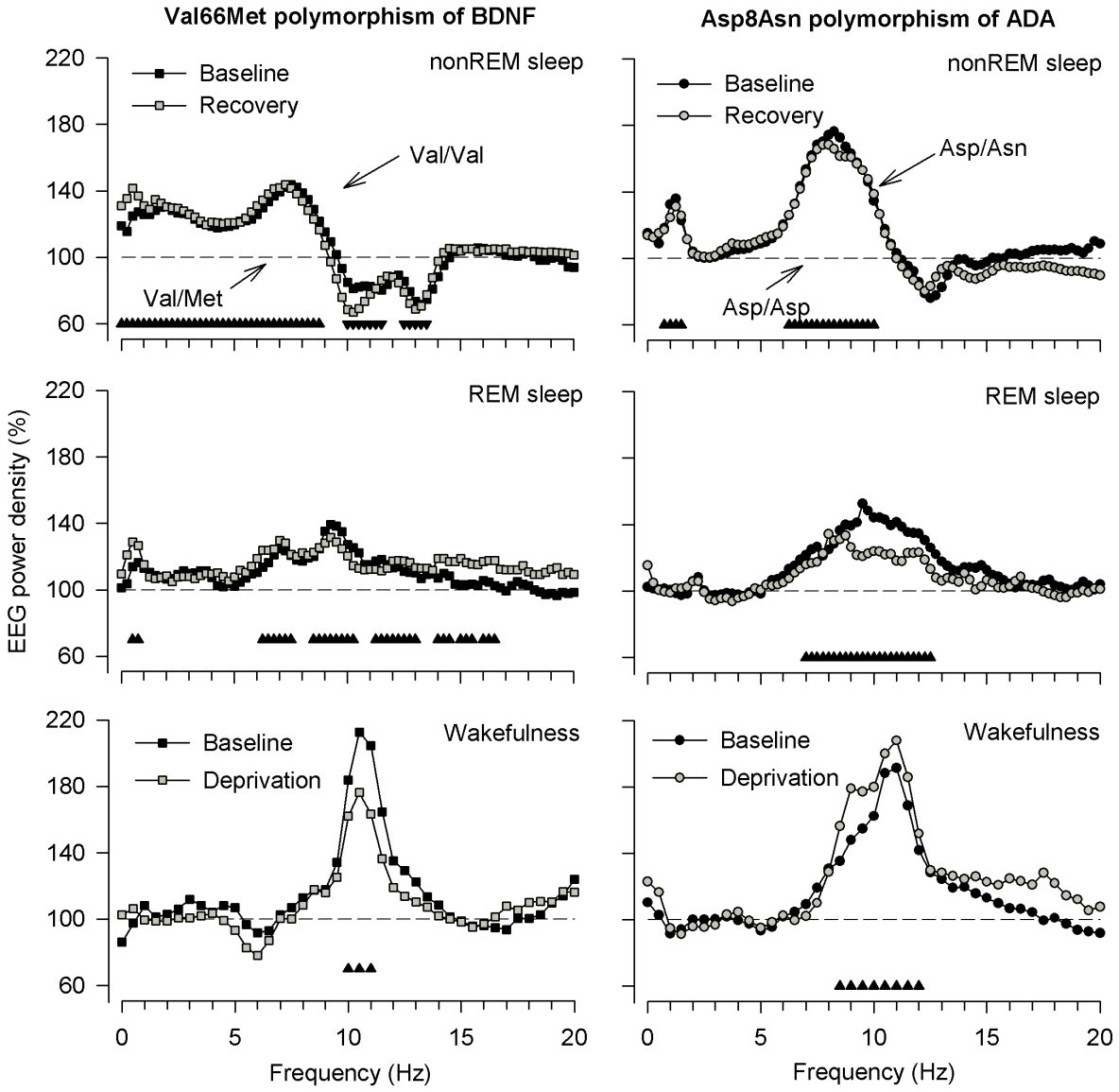

Figure 2: The functional p.Val66Met polymorphism of brain-derived neurotrophic factor (*BDNF*) (left panel) and the functional p.Asp8Asn polymorphism of the adenosine deaminase (*ADA*) (right panel) modulate EEG activity in nonREM sleep, REM sleep and wakefulness in a similar way in baseline and recovery night

(left panel) EEG power density (C3A2 derivation) between 0-20 Hz in the Val/Met genotype as expressed as a percentage of the corresponding values in the Val/Val genotype. The horizontal line corresponds to 100% of the Val/Met genotype. **(right panel)** EEG power density (C3A2 derivation) between 0-20 Hz in the Asp/Asn genotype was expressed as a percentage of the corresponding values in the Asp/Asp genotype. The horizontal line corresponds to 100% of the Asp/Asp genotype.
Data in nonREM (stages 2,3,4) and REM sleep represent all-night values in baseline (black symbols) and recovery nights (grey symbols). In the waking EEG, average power four 5-min recordings at 8 am, 11 am, 2 pm, 5 pm and 8 pm on day 1 (baseline, black squares) and day 2 (deprivation, grey squares) during prolonged wakefulness were analyzed. Geometric means are plotted for each 0.25 Hz bin in nonREM and REM sleep, and for each 0.5 Hz bin in wakefulness. Black triangles denote a significant effect ($p<0.05$) of 'genotype' (either Val/Val, Val/Met or Asp/Asn, Asp/Asp) and 'condition' (baseline, recovery/deprivation). ANOVA revealed no significant 'genotype' x 'condition' interaction.

6.3. Are differences in EEG alpha activity predictive of subjective sleepiness and cognitive performance?

Genetic polymorphisms differently contribute to the modulation of alpha activity in nonREM sleep, REM sleep and wakefulness (Bodenmann, 2009, Rétey et al., 2005, Bachmann et al. submitted). We suggest, that genotype-dependent differences in alpha activity might be predictive for waking quality and cognitive performance. This is in line with a study suggesting that upper oscillations (10-12 Hz) are supposed to play an important role in working memory processes (Klimesch, 1999). Our data demonstrate that working memory performance is not only modulated by the p.Val66Met polymorphism of *BDNF*, but also by the p.Val158Met polymorphism of *COMT* during rested and sleep-deprived state and both polymorphisms also show a genotype-dependent difference in the alpha frequency range roughly above 10 Hz. In addition, subjective sleepiness was not affected by the genotypes. Therefore, we hypothesize that alpha activity above 10 Hz is predictive for working memory processes, but not for subjective sleepiness. This assumption is in line with early findings on alpha activity and cognitive performance suggesting that distinct frequency ranges within the broad alpha range are associated with individual differences cognitive processes (Klimesch, 1996).

In contrast to these effects of the *BDNF* and *COMT* genotype on alpha activity above 10 Hz, the p.Asp8Asn polymorphism of *ADA* and the polymorphism of *ADORA2A* contribute to differences in alpha activity below 10 Hz. These differences in theta/alpha activity may predict differences in vigilant attention (Oken et al., 2006). Consistent with this notion, we found that the functional p.Asp8Asn polymorphism of *ADA* and the functional polymorphism *ADORA2A* modify vigilant attention throughout sleep deprivation, as measured by the PVT (Bodenmann, 2009, Bachmann et al., in prep.). Remarkably, the differences in reaction times between Asp/Asn allele carriers of the *ADA* genotype and between homozygous Asp allele carriers were large, comparable to the impairment of performance after one night without sleep. In addition, the former also performed worse in the d2 attention task than the latter, which indicates that differences in vigilant attention are robust and reliable. The question arises whether impaired vigilant attention is associated with increased subjective sleepiness during 40 hours of prolonged wakefulness. Higher subjective sleepiness was found in Asp/Asn genotype than Asp/Asp genotype subjects.

However, the *ADORA2A* polymorphism did not predict differences in subjective sleepiness. Interestingly, measures of subjective sleepiness in healthy adults have been shown to reflect increased levels of alpha activity during waking EEG recordings with eyes open (Torsvall and Åkerstedt, 1987, Åkerstedt and Gillberg, 1990).

Taken together, neuronal mechanisms, which are involved in the generation of alpha activity are differently manipulated by the studied candidate genes. Thus, we suggest, that the generation of alpha activity in healthy individuals might be regulated by multiple neuronal systems, which are in part genetically determined (van Beijsterveldt et al., 1996). We speculate that alpha frequencies above 10 Hz are predictive of working memory processes, whereas alpha frequencies below 10 Hz are associated with sustained vigilant attention and inter-individual differences in subjective sleepiness. Thus, differences in alpha frequency might be a predictor of working memory performance and vigilant attention.

6.4. Perspectives

In conclusion, the results of the current thesis provide new indications to understand the neuronal basis of trait-like differences in human cognitive performance and physiological sleep-wake regulation. The thesis shows that the identification of candidate genes contributing to trait-like inter-individual differences in cognitive and sleep-wake regulatory mechanisms is important to provide new insights into the neurobiology of sleep and cognitive performance. We could show in healthy humans that the mechanisms that mediate the effects of cognitive performance including impaired performance after sleep deprivation, seem to be different to some extent from those that mediate the effects of sleep deprivation on sleep physiology. The studies introduced in the present thesis increase our understanding of the different neuronal systems involved in cognitive and sleep-wake regulatory processes. This basic knowledge is crucial for future treatments of neurological diseases, which are often associated with impaired cognitive abilities and disrupted sleep.

Table 1: Synopsis of the main results of the present thesis

Context:	Neuronal networks of numerical cognition **Chapter 2**	Cognitive abilities and habitual sleep duration **Chapter 3**	Genes contributing to sleep-wake regulation **Chapter 4**	Genes contributing to sleep-wake regulation **Chapter 5**
Topic:	**Spatial-numerical interactions involving the prefrontal cortex**	**Executive functioning and habitual sleep duration**	**p.Val66Met polymorphism of BDNF in sleep-wake regulation**	**p.Asp8Asn polymorphism of ADA in sleep-wake regulation**
Cognitive testing & effects:	<u>SNB:</u> Design fluency (right hemisphere dominance) > Letter fluency (left hemisphere dominance) <u>no SNB:</u> Letter fluency (left hemisphere dominance) > Design fluency (right hemisphere dominance) Asymmetries in learning & memory not related to SNB	<u>Interference/inhibition control</u> Subjective & objective sd: sd HFP < sd LFP (Stroop Color-Word task, n=220 & n=82) <u>Response stereotypy</u> Subjective sd: sd HFP < sd LFP (adjacency on RNG task, n=220 & n=82) <u>Maintenance variability</u> Subjective sd: sd HFP < sd LFP (d2 task, n=220 & n=82) <u>Learning & memory:</u> sd HFP = sd LFP	<u>Sustained attention</u> • Val/Met = Val/Val (PVT, n=22) • Impairment of SD: Val/Met = Val/Val <u>Working memory</u> Response accuracy (2-back task, n=22): • Val/Met < Val/Val • Similar in bl & SD Impairment SD: • Val/Met = Val/Val	<u>Sustained attention</u> • Asp/Asn < Asp/Asp (d2 task, n=220 & n=22) • Asp/Asn< Asp/Asp (PVT, n=22) • Similar in bl & SD Impairment of SD: • Asp/Asn= Asp/Asp <u>Working memory</u> • Asp/Asn= Asp/Asp (2-back task, n=22) Impairment SD: • Asp/Asn= Asp/Asp
Subjective effects:		<u>ESS (n=220 & n=82)</u> HFP = LFP <u>Education level (n=220 & n=82)</u> HFP = LFP	<u>SSS & Bf-S (n=22)</u> Subjective sleepiness & well-being: • Val/Met = Val/Val Impairment SD: • Val/Met = Val/Val	<u>SSS & POMS (fatigue & vigor) (n=22)</u> • Asp/Asn sleepier • Asp/Asn reduced vigor • Similar in bl & SD Impairment SD: • Asp/Asn = Asp/Asp

Topic:	Spatial-numerical interactions involving the prefrontal cortex	Executive functioning and habitual sleep duration	p.Val66Met polymorphism of BDNF in sleep-wake regulation	p.Asp8Asn polymorphism of ADA in sleep-wake regulation
Habitual sleep duration: **Habitual sleep duration:**		MCTQ (n=220 & n=82) sd women > sd men sd week < sd leisure days 4-week actigraphy/sleep diary sd week < sd leisure days (n=82) MCTQ vs. actimetry: Leisure days: sd MCTQ > sd actimetry Word days: sd MCTQ = sd actimetry	MCTQ (n=22) Sd Val/Val = sd Val/Met (Work & leisure days)	MCTQ (n=220 & n=22) Sd Asp/Asn = sd Asp/Asp (Work & leisure days) 4-week actigraphy (n=20) Sd Asp/Asn = sd Asp/Asp (Work & leisure days)
Sleep architecture:			Stage 4 in bl & rec: • Val/Met < Val/Val Stage 2 in bl & rec: • Val/Met > Val/Val Impairment SD: • Val/Met = Val/Val	SWS in bl & rec: • Asp/Asn > Asp/Asp Stage 2 in bl & rec: • Asp/Asn< Asp/Asp Impairment SD: • Asp/Asn= Asp/Asp
EEG Wakefulness			Alpha activity (10 - 11.5 Hz) • Val/Met < Val/Val • Similar in bl & SD • Theta activity (5 - 8 Hz) • Val/Met = Val/Val Impairment SD: • Val/Met = Val/Val	Alpha activity (8.5 - 12 Hz) • Asp/Asn > Asp/Asp • Similar in bl & SD • Theta activity (5- 8 Hz) • Asp/Asn = Asp/Asp Impairment SD: • Asp/Asn = Asp/Asp

Topic:	Spatial-numerical interactions involving the prefrontal cortex	Executive functioning and habitual sleep duration	p.Val66Met polymorphism of BDNF in sleep-wake regulation	p.Asp8Asn polymorphism of ADA in sleep-wake regulation
EEG nonREM sleep (stages 2-4)			<u>Delta activity (0.0 - 8.75 Hz)</u> • Val/Met < Val/Val Build-up SWA (0.75 – 4.5 Hz): • Val/Met < Val/Val Dynamics sleep homeostasis (time constant τ: • Val/Met = Val/Val <u>Alpha/Sigma activity (10 - 11.5 Hz / 12.5 - 13.5 Hz)</u> • Val/Met > Val/Val • Similar in bl & rec Impairment SD: • Val/Met = Val/Val	<u>Delta activity (0.75 - 1.5 Hz)</u> • Asp/Asn > Asp/Asp Dynamics sleep homeostasis: • Asp/Asn = Asp/Asp <u>Theta/alpha activity (6.5 - 10 Hz)</u> • Asp/Asn > Asp/Asp • Similar in bl & rec Impairment SD: Asp/Asn = Asp/Asp
EEG REM sleep			<u>Alpha activity (6.25 - 15.5 Hz)</u> • Val/Met > Val/Val • Similar in bl & rec Impairment SD: • Val/Met = Val/Val	<u>Theta/alpha activity (7 - 12.5 Hz)</u> • Asp/Asn > Asp/Asp • Similar in bl & rec Impairment SD: • Asp/Asn = Asp/Asp
α-Amylase activity				• Asp/Asn > Asp/Asp • Similar in bl & SD Impairment SD: • Asp/Asn = Asp/Asp

ADA: Adenosine deaminase; **Asn**: Asparagine; **Asp**: Aspartic acid; **BDNF**: Brain-derived neurotrophic factor; **Bf-S**: Befindlichkeits-Skala; **bl:** baseline; **ESS:** Epworth sleepiness scale; **HFP:** higher-functioning performers; **LFIP** lower-functioning performer; **MCTQ:** Munich Chronotype Questionnaire; **Met**: Methionine; **POMS:** Profile of Mood States; **PVT:** Psychomotor vigilance task; **RAVLT:** Rey auditory visual learning task; **rec:** Recovery; **RNG:** random number generation Task; **RVDLT:** Rey verbal design learning task; **SD:** Sleep deprivation; **sd:** sleep duration; **SNB:** small number bias; **SSS:** Subjective sleepiness scale; **SWA:** Slow-wave-activity; **SWS:** Slow wave sleep; **Val**: Valine.

References

Acebo C, LeBourgeois MK. (2006). Actigraphy. *Respir Care Clin N Am*, 12(1), 23-30.

Achermann P. (2004). The two-process model of sleep regulation revisited. *Aviat Space Environ Med*, 75(3 Suppl), A37-43.

Achermann P, Borbély AA. (1997). Low-frequency (< 1 Hz) oscillations in the human sleep EEG. *Neuroscience*, 81, 213-222.

Adam M, Rétey JV, Khatami R, Landolt HP. (2006). Age-related changes in the time course of vigilant attention during 40 hours without sleep in men. *Sleep*, 29, 55-57.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. (2007). Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*, 450(7168), 420-424.

Aeschbach D, Cajochen C, Landolt HP, Borbély AA. (1996). Homeostatic sleep regulation in habitual short sleepers and long sleepers. *Am J Physiol*, 270(1), R41-R53.

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, Giesen HA, Wehr TA. (2000). Short sleepers tolerate a higher homeostatic sleep pressure than long sleepers: evidence from the waking electroencephalogram. *Sleep*, 23, A34.

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, Wehr TA. (2001). Evidence from the waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure than long sleepers. *Neuroscience*, 102(3), 493-502.

Aguilar-Roblero R, Verduzco-Carbajal L, Rodriguez C, Mendez-Franco J, Moran J, Perez de la Mora M. (1993). Circadian rhythmicity in the GABAergic system in the suprachiasmatic nuclei of the rat. *Neuroscience Letters*, 157, 199-202.

Akerstedt T, Gillberg M. (1990). Subjective and objective sleepiness in the active individual. *Int J Neurosci*, 52(1-2), 29-37.

Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. (2010). Caffeine prevents sleep loss-induced deficits in long-term potentiation and related signaling molecules in the dentate gyrus. *Eur J Neurosci*, 31(8), 1368-1376.

Allebrandt KV, Roenneberg T. (2008). The search for circadian clock components in humans: new perspectives for association studies. *Braz J Med Biol Res*, 41(8), 716-721.

Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Muller-Myhsok B, Pramstaller P, Merrow M, Meitinger T, Metspalu A, Roenneberg T. (2010). CLOCK gene variants associate with sleep duration in two independent populations. *Biol Psychiatry*, 67(11), 1040-1047.

Ambrosius U, Lietzenmaier S, Wehrle R, Wichniak A, Kalus S, Winkelmann J, Bettecken T, Holsboer F, Yassouridis A, Friess E. (2008). Heritability of sleep electroencephalogram. *Biol Psychiatry*, 64, 344-348.

Amzica F, Steriade M. (1997). The K-complex - its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology*, 49(4), 952-959.

Amzica F, Steriade M. (2002). The functional significance of K-complexes. *Sleep Med Rev*, 6(2), 139-149.

Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. (2003). The role of actigraphy in the study of sleep and circadian rhythms. *Sleep*, 26(3), 342-392.

Andretic R, Franken P, Tafti M. (2008). Genetics of sleep. *Annu Rev Genet*, 42, 361-388.

Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, von Schantz M. (2003). A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep*, 26(4), 413-415.

Aserinsky E, Kleitman N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*, 118, 273-274.

Assaife-Lopes N, Sousa VC, Pereira DB, Ribeiro JA, Chao MV, Sebastiao AM. (2010). Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF-mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. *J Neurosci*, 30(25), 8468-8480.

Aston-Jones G, Bloom FE. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci*, 1(8), 876-886.

Bachmann V, Klaus F, Bodenmann S, Schäfer N, Brugger P, Huber S, Berger W, Landolt HP. (2011). The functional Asp8Asn polymorphism of adenosine deaminase (ADA) predicts inter-indivdual differences in homeostatic sleep pressure. (submitted).

Backhaus J, Hoeckesfeld R, Born J, Hohagen F, Junghanns K. (2008). Immediate as well as delayed post learning sleep but not wakefulness enhances declarative memory consolidation in children. *Neurobiol Learn Mem*, 89(1), 76-80.

Baddeley A, Emslie H, Kolodny J, Duncan J. (1998). Random generation and the executive control of working memory. *Q J Exp Psychol A*, 51(4), 819-852.
Baldo JV, Shimamura AP, Delis DC, Kramer J, Kaplan E. (2001). Verbal and design fluency in patients with frontal lobe lesions. *J. Int. Neuropsychol. Soc.*, 7(5), 586-596.
Barker PA. (2009). Whither proBDNF? *Nat Neurosci*, 12(2), 105-106.
Basheer R, Bauer A, Elmenhorst D, Ramesh V, McCarley RW. (2007). Sleep deprivation upregulates A1 adenosine receptors in the rat basal forebrain. *Neuroreport*, 18(18), 1895-1899.
Basheer R, Strecker RE, Thakkar MM, McCarley RW. (2004). Adenosine and sleep-wake regulation. *Prog Neurobiol*, 73, 379-396.
Bath KG, Lee FS. (2006). Variant BDNF (Val66Met) impact on brain structure and function. *Cogn Affect Behav Neurosci*, 6(1), 79-85.
Battistuzzi G, Iudicone P, Santolamazza P, Petrucci R. (1981). Activity of adenosine deaminase allelic forms in intact erythrocytes and in lymphocytes. *Ann Hum Genet*, 45(Pt 1), 15-19.
Bauer A, Ishiwata K. (2009). Adenosine receptor ligands and PET imaging of the CNS. *Handb Exp Pharmacol*(193), 617-642.
Benford F. (1938). The law of anomalous numbers. *Proceedings of the American Philosophical Society*, 78(4), 551-572.
Benington JH, Heller HC. (1995). Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol*, 45, 347-360.
Berger H. (1929). Über das Elektroenzephalogramm des Menschen. *Arch. Psychiat. Nervenkr.*, 87, 527-570.
Binder DK, Scharfman HE. (2004). Brain-derived neurotrophic factor. *Growth Factors*, 22(3), 123-131.
Binks PG, Waters WF, Hurry M. (1999). Short-term total sleep deprivations does not selectively impair higher cortical functioning. *Sleep*, 22(3), 328-334.
Bjorness TE, Kelly CL, Gao T, Poffenberger V, Greene RW. (2009). Control and function of the homeostatic sleep response by adenosine A1 receptors. *J Neurosci*, 29(5), 1267-1276.
Bliss TV, Lomo T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol*, 232(2), 331-356.
Bodenmann S. (2009). Sleep-wake regulation in humans: pharmacogenetic studies. *Dissertation*.
Bodenmann S, Rusterholz T, Durr R, Stoll C, Bachmann V, Geissler E, Jaggi-Schwarz K, Landolt HP. (2009a). The functional Val158Met polymorphism of COMT predicts interindividual differences in brain alpha oscillations in young men. *J Neurosci*, 29(35), 10855-10862.
Bodenmann S, Xu S, Luhmann UF, Arand M, Berger W, Jung HH, Landolt HP. (2009b). Pharmacogenetics of modafinil after sleep loss: catechol-O-methyltransferase genotype modulates waking functions but not recovery sleep. *Clin Pharmacol Ther*, 85(3), 296-304.
Bodizs R, Kis T, Lazar AS, Havran L, Rigo P, Clemens Z, Halasz P. (2005). Prediction of general mental ability based on neural oscillation measures of sleep. *J Sleep Res*, 14(3), 285-292.
Bongard S, Nieder A. (2010). Basic mathematical rules are encoded by primate prefrontal cortex neurons. *Proc Natl Acad Sci U S A*, 107(5), 2277-2282.
Borbély, Acherman P (2000) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine(Kryger, M. H. et al., eds), pp 377-390 Philadelphia, PA: W.B. Saunders Company.
Borbely AA. (1982). A two process model of sleep regulation. *Hum Neurobiol*, 1(3), 195-204.
Borbély AA. (1984). Schlafgewohnheiten,Schlafqualität und Schlafmittelkonsum der Schweizer Bevölkerung. Ergebnisse einer Repräsentativumfrage. *Schweizerische Ärztezeitung*, 65, 1606-1613.
Borbély AA, Achermann P (2005) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine(Kryger, M. H. et al., eds), pp 405-417 Philadelphia, PA: Elsevier Saunders.
Borroni AM, Fichtenholtz H, Woodside BL, Teyler TJ. (2000). Role of voltage-dependent calcium channel long-term potentiation (LTP) and NMDA LTP in spatial memory. *J Neurosci*, 20(24), 9272-9276.
Bottini G, Toraldo A. (2003). The influence of contralesional targets on the cancellation of ipsilesional targets in unilateral neglect. *Brain Cogn*, 53(2), 117-120.
Bowers D, Heilman KM. (1980). Pseudoneglect: effects of hemispace on a tactile line bisection task. *Neuropsychologia*, 18(4-5), 491-498.
Bradshaw JL, Nathan G, Nettleton NC, Wilson L, Pierson J. (1987). Why is there a left side underestimation in rod bisection? *Neuropsychologia*, 25(4), 735-738.
Bradshaw JL, Nettleton NC, Nathan G, Wilson L. (1983). Head and body space to left and right, front and rear--II. Visuotactual and kinesthetic studies and left-side underestimation. *Neuropsychologia*, 21(5), 475-486.
Bremer F. (1935). Cereveau isole et physiologie du sommeil. *CR Soc Biol (Paris)*, 118, 1235-1242.

Brickenkamp R. (1962). Aufmerksamkeits-Belastungs-Test (Test d2). [The d2 test of Attention]. *Göttingen:Hogrefe*.

Brugger P, Monsch AU, Johnson SA. (1996a). Repetitive behavior and repetition avoidance: the role of the right hemisphere. *J Psychiatry Neurosc.*, 21(1), 53-56.

Brugger P, Monsch AU, Salmon DP, Butters N. (1996b). Random number generation in dementia of the Alzheimer type: a test of frontal executive functions. *Neuropsychologia*, 34(2), 97-103.

Brugger P, Schubiger M, Loetscher T. (2010). Leftward bias in number space is modulated by magical ideation. *Cogn Behav Neurol*, 23(2), 119-123.

Brunoni AR, Lopes M, Fregni F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*, 11(8), 1169-1180.

Buchmann A, Ringli M, Kurth S, Schaerer M, Geiger A, Jenni OG, Huber R. (2010). EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation. *Cereb Cortex*.

Buckelmüller J, Landolt HP, Stassen HH, Achermann P. (2006). Trait-like individual differences in the human sleep electroencephalogram. *Neuroscience*, 138(1), 351-356.

Burns B. (2009). Sensitivity to statistical regularities: People (largely) follow Benford's law. *Proceedings of the Cognitive Science Societey*, 637.

Cajochen C, Brunner DP, Kräuchi K, Graw P, Wirz-Justice A. (1995). Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. *Sleep*, 18(10), 890-894.

Cajochen C, Foy R, Dijk DJ. (1999). Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Research Online*, 2(3), 65-69.

Cajochen C, Knoblauch V, Kräuchi K, Renz C, Wirz-Justice A. (2001). Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. *Neuroreport*, 12(10), 2277-2281.

Cajochen C, Wyatt JK, Czeisler CA, Dijk DJ. (2002). Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience*, 114(4), 1047-1060.

Cantlon JF, Libertus ME, Pinel P, Dehaene S, Brannon EM, Pelphrey KA. (2009). The neural development of an abstract concept of number. *J Cogn Neurosci*, 21(11), 2217-2229.

Carvalho AL, Caldeira MV, Santos SD, Duarte CB. (2008). Role of the brain-derived neurotrophic factor at glutamatergic synapses. *Br J Pharmacol*, 153 Suppl 1, 310-324.

Casasanto D. (2009). Embodiment of abstract concepts: good and bad in right- and left-handers. *J Exp Psychol Gen*, 138(3), 351-367.

Chamberlin NL, Arrigoni E, Chou TC, Scammell TE, Greene RW, Saper CB. (2003). Effects of adenosine on GABAergic synaptic inputs to identified ventrolateral preoptic neurons. *Neuroscience*, 119(4), 913-918.

Chapman LJ, Chapman JP. (1987). The measurement of handedness. *Brain Cogn*, 6(2), 175-183.

Chee MW, Choo WC. (2004). Functional imaging of working memory after 24 hr of total sleep deprivation. *J Neurosci*, 24(19), 4560-4567.

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, 314(5796), 140-143.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS. (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci*, 24(18), 4401-4411.

Cirelli C. (2002). How sleep deprivation affects gene expression in the brain: a review of recent findings. *J Appl Physiol*, 92(1), 394-400.

Cirelli C, Tononi G. (2000). Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. *J Neurosci*, 20(24), 9187-9194.

Contreras D, Steriade M. (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci*, 15, 604-622.

Contreras D, Steriade M. (1996). Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. *J Physiol*, 490, 159-179.

Cooley WJ, Tukey JW. (1965). An algorithm for the machine calculation of complex Fourier series. *Math Comput*, 19, 297-301.

Crocker A, Sehgal A. (2010). Genetic analysis of sleep. *Genes Dev*, 24(12), 1220-1235.

Cunha C, Brambilla R, Thomas KL. (2010). A simple role for BDNF in learning and memory? *Front Mol Neurosci*, 3, 1.

Daniels C, Witt K, Wolff S, Jansen O, Deuschl G. (2003). Rate dependency of the human cortical network subserving executive functions during generation of random number series--a functional magnetic resonance imaging study. *Neurosci Lett*, 345(1), 25-28.

de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG. (2004). COMT gene polymorphism is associated with declarative memory in adulthood and old age. *Behav Genet*, 34(5), 533-539.

De Gennaro L, Marzano C, Fratello F, Moroni F, Pellicciari MC, Ferlazzo F, Costa S, Couyoumdjian A, Curcio G, Sforza E, Malafosse A, Finelli L, Pasqualetti P, Ferrara M, Bertini M, Rossini PM. (2008). The EEG fingerprint of sleep is genetically determined: a twin study. *Ann Neurol*, 64(4), 455-460.

Dehaene S (1997) The number sense. How the mind creates mathematics: Oxford: Oxford University Press.

Dehaene S, Bossini S, Giraux P. (1993). The mental representation of parity and number magnitude. *J Exp Psychol Gen*, 122, 371-396.

Dehaene S, Mehler J. (1992). Cross-linguistic regularities in the frequency of number words. *Cognition*, 43(1), 1-29.

Dement W, Kleitman N. (1957). Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. *EEG Clin Neurophysiol*, 9, 673-690.

Dempster E, Toulopoulou T, McDonald C, Bramon E, Walshe M, Filbey F, Wickham H, Sham PC, Murray RM, Collier DA. (2005). Association between BDNF Val(66)Met genotype and episodic memory. *Am J Med Genet B Neuropsychiatr Genet*, 134B(1), 73-75.

Diekamp B, Regolin L, Gunturkun O, Vallortigara G. (2005). A left-sided visuospatial bias in birds. *Curr Biol*, 15(10), 372-373.

Diekmann T. (2007). Not the first digit! Using Benford's Law to detect fraudulent scientific data. *J Appl Stat*, 34(3), 321-329.

Dijk DJ, Archer SN. (2010). PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev*, 14(3), 151-160.

Dijk DJ, Czeisler CA. (1994). Paradoxical Timing of the Circadian-Rhythm of Sleep Propensity Serves to Consolidate Sleep and Wakefulness in Humans. *Neuroscience Letters*, 166(1), 63-68.

Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, Aptowicz C, Pack AI. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep*, 20(4), 267-277.

Doricchi F, Guariglia P, Gasparini M, Tomaiuolo F. (2005). Dissociation between physical and mental number line bisection in right hemisphere brain damage. *Nat Neurosci*, 8(12), 1663-1665.

Doricchi F, Merola S, Aiello M, Guariglia P, Bruschini M, Gevers W, Gasparini M, Tomaiuolo F. (2009). Spatial orienting biases in the decimal numeral system. *Curr Biol*, 19(8), 682-687.

Dorrian J, Rogers NL, Dinges DF (2005) Psychomotor vigilance performance: a neurocognitive assay sensitive to sleep loss. In: Sleep Deprivation: Clinical Issues, Pharmacology and Sleep Loss Effects (Kushida, C., ed), pp 39-70 New York: Marcel Dekker, Inc.

Drummond SP, Bischoff-Grethe A, Dinges DF, Ayalon L, Mednick SC, Meloy MJ. (2005). The neural basis of the psychomotor vigilance task. *Sleep*, 28(9), 1059-1068.

Drummond SP, Brown GG, Gillin JC, Stricker JL, Wong EC, Buxton RB. (2000). Altered brain response to verbal learning following sleep deprivation. *Nature*, 403(6770), 655-657.

Drummond SP, Brown GG, Stricker JL, Buxton RB, Wong EC, Gillin JC. (1999). Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport*, 10(18), 3745-3748.

Duncan J, Seitz RJ, Kolodny J, Bor D, Herzog H, Ahmed A, Newell FN, Emslie H. (2000). A neural basis for general intelligence. *Science*, 289(5478), 457-460.

Durmer JS, Dinges DF. (2005). Neurocognitive consequences of sleep deprivation. *Semin Neurol*, 25(1), 117-129.

Ebersbach G, Trottenberg T, Hattig H, Schelosky L, Schrag A, Poewe W. (1996). Directional bias of initial visual exploration. A symptom of neglect in Parkinson's disease. *Brain*, 119 (Pt 1), 79-87.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. (2001). Effect of COMT Val(108/158) Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA*, 98(12), 6917-6922.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112(2), 257-269.

Elmenhorst D, Meyer PT, Matusch A, Winz OH, Zilles K, Bauer A. (2007). Test-retest stability of cerebral A(1) adenosine receptor quantification using [F-18]CPFPX and PET. *Eur J Nucl Med Mol Imaging*, 34(7), 1061-1070.

Ertel S (2005) The ball drawing test: Psi from untrodden ground. In: Parapsychology in the Twenty-First Century: Essays on the Future of Parapsychological Research (Thalbourne, M. A. S., L., ed), pp 90-123 Jefferson NC: McFarland.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C. (2008). A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J Neurosci*, 28(15), 4088-4095.

Faubel R, Lopez-Garcia E, Guallar-Castillon P, Graciani A, Banegas JR, Rodriguez-Artalejo F. (2009). Usual sleep duration and cognitive function in older adults in Spain. *J Sleep Res*, 18(4), 427-435.

Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG. (2009). Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci U S A*, 106(35), 15037-15042.

Fifer WP, Byrd DL, Kaku M, Eigsti IM, Isler JR, Grose-Fifer J, Tarullo AR, Balsam PD. (2010). Newborn infants learn during sleep. *Proc Natl Acad Sci U S A*.

Finelli LA, Acherman P, Borbely AA. (2001a). Individual 'fingerprints' in human sleep EEG topography. *Neuropsychopharmacology*, 25, 57-62.

Finelli LA, Borbély AA, Achermann P. (2001b). Functional topography of the human nonREM sleep electroencephalogram. *Eur J Neurosci*, 13, 2282-2290.

Fischer MH, Mills RA, Shaki S. (2010). How to cook a SNARC: Number placement in text rapidly changes spatial-numerical associations. *Brain Cogn*, 72(3), 333-336.

Folstein MF, Folstein SE, McHugh PR. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, 12(3), 189-198.

Fontinha BM, Diogenes MJ, Ribeiro JA, Sebastiao AM. (2008). Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. *Neuropharmacology*, 54(6), 924-933.

Fort P, Bassetti CL, Luppi PH. (2009). Alternating vigilance states: new insights regarding neuronal networks and mechanisms. *Eur J Neurosci*, 29(9), 1741-1753.

Foster PS, Drago V, Harrison DW. (2009). Assessment of nonverbal learning and memory using the Design Learning Test. *J Psychol*, 143(3), 245-266.

Franken P, Chollet D, Tafti M. (2001). The homeostatic regulation of sleep need is under genetic control. *J Neurosci*, 21(8), 2610-2621.

Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. (2000). Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch Pharmacol*, 362(4-5), 364-374.

Fredholm BB, Bättig K, Holmen J, Nehlig A, Zvartau EE. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*, 51(1), 83-133.

Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. (2005a). Adenosine and brain function. *Int Rev Neurobiol*, 63, 191-270.

Fredholm BB, Chen JF, Masino SA, Vaugeois JM. (2005b). Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs. *Annu Rev Pharmacol Toxicol*, 45, 385-412.

Fukunaga K, Miyamoto E. (2000). A working model of CaM kinase II activity in hippocampal long-term potentiation and memory. *Neurosci Res*, 38(1), 3-17.

Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P. (2005). The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. *Neuroscience*, 134(4), 1377-1390.

Gaus SE, Strecker RE, Tate BA, Parker RA, Saper CB. (2002). Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. *Neuroscience*, 115(1), 285-294.

Gazzaniga MS, Ivri RB, Mangun GR (2002) Cognitive Neuroscience: the biology of the mind. New York: W.W.Norton & Company, Inc.

Geiger A, Achermann P, Jenni OG. (2010). Association between sleep duration and intelligence scores in healthy children. *Dev Psychol*, 46(4), 949-954.

Gelman R, Gallistel CR (1978) The child's understanding of number: Cambridge Mass: Harward University Press.

Genoud C, Knott GW, Sakata K, Lu B, Welker E. (2004). Altered synapse formation in the adult somatosensory cortex of brain-derived neurotrophic factor heterozygote mice. *J Neurosci*, 24(10), 2394-2400.

Gobel SM, Calabria M, Farne A, Rossetti Y. (2006). Parietal rTMS distorts the mental number line: simulating 'spatial' neglect in healthy subjects. *Neuropsychologia*, 44(6), 860-868.

Goel N, Rao H, Durmer JS, Dinges DF. (2009). Neurocognitive consequences of sleep deprivation. *Semin Neurol*, 29(4), 320-339.

Golby AJ, Poldrack RA, Brewer JB, Spencer D, Desmond JE, Aron AP, Gabrieli JD. (2001). Material-specific lateralization in the medial temporal lobe and prefrontal cortex during memory encoding. *Brain*, 124, 1841-1854.

Gomez RL, Bootzin RR, Nadel L. (2006). Naps promote abstraction in language-learning infants. *Psychol Sci*, 17(8), 670-674.

Gottselig JM, Adam M, Rétey JV, Khatami R, Achermann P, Landolt H-P. (2006). Random number generation during sleep deprivation: Effects of caffeine on response maintenance and stereotypy. *J Sleep Res*, 15, 31-40.

Greene CM, Robertson IH, Gill M, Bellgrove MA. (2010). Dopaminergic genotype influences spatial bias in healthy adults. *Neuropsychologia*, 48(9), 2458-2464.

Greenwald AG, McGhee DE, Schwartz JL. (1998). Measuring individual differences in implicit cognition: the implicit association test. *J Pers Soc Psychol*, 74(6), 1464-1480.

Groeger JA, Zijlstra FR, Dijk DJ. (2004). Sleep quantity, sleep difficulties and their perceived consequences in a representative sample of some 2000 British adults. *J Sleep Res*, 13(4), 359-371.

Gruber R, Laviolette R, Deluca P, Monson E, Cornish K, Carrier J. (2010). Short sleep duration is associated with poor performance on IQ measures in healthy school-age children. *Sleep Med*, 11(3), 289-294.

Gunturkun O. (1997). Visual lateralization in birds: from neurotrophins to cognition? *Eur J Morphol*, 35(4), 290-302.

Hairston IS, Peyron C, Denning DP, Ruby NF, Flores J, Sapolsky RM, Heller HC, O'Hara BF. (2004). Sleep deprivation effects on growth factor expression in neonatal rats: a potential role for BDNF in the mediation of delta power. *J Neurophysiol*, 91(4), 1586-1595.

Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG. (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*, 61(2), 213-219.

Halassa MM, Haydon PG. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*, 72, 335-355.

Hallanger AE, Levey AI, Lee HJ, Rye DB, Wainer BH. (1987). The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J Comp Neurol*, 262(1), 105-124.

Hanlon EC, Faraguna U, Vyazovskiy VV, Tononi G, Cirelli C. (2009). Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. *Sleep*, 32(6), 719-729.

Happe S, Klosch G, Lorenzo J, Kunz D, Penzel T, Roschke J, Himanen SL, Gruber G, Zeitlhofer J. (2005). Perception of sleep: subjective versus objective sleep parameters in patients with Parkinson's disease in comparison with healthy elderly controls. Sleep perception in Parkinson's disease and controls. *J Neurol*, 252(8), 936-943.

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. (2003). Brain-derived neurotrophic factor val(66)met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci*, 23(17), 6690-6694.

Harrison Y, Horne JA. (1996). Sleep loss affects frontal lobe function, as shown in complex "real world" tasks. *Sleep Res*, 25, 467.

Harrison Y, Horne JA. (1997). Sleep deprivation affects speech. *Sleep*, 20, 871-877.

Harrison Y, Horne JA, Rothwell A. (2000). Prefrontal neuropsychological effects of sleep deprivation in young adults--a model for healthy aging? *Sleep*, 23(8), 1067-1073.

Hassainia F, Petit D, Nielsen T, Gauthier S, Montplaisir J. (1997). Quantitative EEG and statistical mapping of wakefulness and REM sleep in the evaluation of mild to moderate Alzheimer's disease. *Eur Neurol*, 37(4), 219-224.

Hättig H. (1992). Die Erfassung der spontanen initialen visuellen Explorationsasymmetrie [The assessment of a spontaneous initial visual exploration asymmetry; description and assessment]. *Zeitschrift für Neuropsychologie*, 1, 52-67.

Hayaishi O, Urade Y, Eguchi N, Huang Z-L. (2004). Genes for prostaglandin D synthase and receptor as well as adenosine A_{2A} receptor are involved in the homeostatic regulation of NREM sleep. *Arch Ital Biol*, 142, 533-539.

Haydon PG, Carmignoto G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev*, 86(3), 1009-1031.

He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL, Jr., Rossner MJ, Nishino S, Fu YH. (2009). The transcriptional repressor DEC2 regulates sleep length in mammals. *Science*, 325(5942), 866-870.

Hellige JB, Marks NL. (2001). Are hemispheric strategy differences independent of the level of performance? *Neuropsychology*, 15(3), 380-395.

Heuer H, Kohlisch O, Klein W. (2005). The effects of total sleep deprivation on the generation of random sequences of key-presses, numbers and nouns. *Q J Exp Psychol A*, 58(2), 275-307.
Hirschhorn R, Yang DR, Israni A. (1994). An Asp8Asn substitution results in the adenosine deaminase (ADA) genetic polymorphism (ADA 2 allozyme): occurrence on different chromosomal backgrounds and apparent intragenic crossover. *Ann Hum Genet*, 58(Pt 1), 1-9.
Hobson JA, McCarley RW, Wyzinski PW. (1975). Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*, 189(4196), 55-58.
Hobson JA, Pace-Schott EF. (2002). The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat Rev Neurosci*, 3(9), 679-693.
Horne JA. (1993). Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. *Br. J. Psychiatry*, 162, 413-419.
Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O. (2005). Adenosine A_{2A}, but not A_1, receptors mediate the arousal effect of caffeine. *Nat Neurosci*, 8(7), 858-859.
Hubbard EM, Piazza M, Pinel P, Dehaene S. (2005). Interactions between number and space in parietal cortex. *Nat Rev Neurosci*, 6(6), 435-448.
Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat Neurosci*, 9(9), 1169-1176.
Huber R, Ghilardi MF, Massimini M, Tononi G. (2004). Local sleep and learning. *Nature*, 430(6995), 78-81.
Huber R, Tononi G, Cirelli C. (2007). Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*, 30(2), 129-139.
Illes P, Klotz KN, Lohse MJ. (2000). Signaling by extracellular nucleotides and nucleosides. *Naunyn Schmiedebergs Arch Pharmacol*, 362(4-5), 295-298.
Itagaki F, Niwa S, Itoh K, Momose T. (1995). Random number generation and the frontal cortex. *Int J Psychophysiol*, 19(1), 79-80.
Jahanshahi M, Dirnberger G, Fuller R, Frith CD. (2000). The role of the dorsolateral prefrontal cortex in random number generation: a study with positron emission tomography. *Neuroimage*, 12(6), 713-725.
Jenni OG, Caflisch JA, Molinari L (2009) A negative relationship between sleep duration and intellectual abilities in children and adolescents. In: Bianual Meeting of the Society of Research in Child Development Denver, CO.
Johns MW. (1991). A New Method for Measuring Daytime Sleepiness - the Epworth Sleepiness Scale. *Sleep*, 14(6), 540-545.
Jones BE (2005a) Basic mechanisms of sleep-wake states. In: Principles and Practise of Sleep Medicine (Kryger, M. H. et al., eds), pp 136-153 Philadelphia, PA: Elsevier Saunders.
Jones BE. (2005b). From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol Sci*, 26(11), 578-586.
Jones BE, Beaudet A. (1987). Distribution of acetylcholine and catecholamine neurons in the cat brainstem: a choline acetyltransferase and tyrosine hydroxylase immunohistochemical study. *J Comp Neurol*, 261(1), 15-32.
Jones K, Harrison Y. (2001). Frontal lobe function, sleep loss and fragmented sleep. *Sleep Med Rev*, 5(6), 463-475.
Kamei S, Morita A, Serizawa K, Mizutani T, Hirayanagi K. (2010). Quantitative EEG analysis of executive dysfunction in Parkinson disease. *J Clin Neurophysiol*, 27(3), 193-197.
Kandel ER, Schwartz JH, Jessel TM (eds.) (2000) Principles of neural science: McGraw-Hill.
Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, Mignot E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep*, 21, 569-576.
Kaufmann L, Koppelstaetter F, Siedentopf C, Haala I, Haberlandt E, Zimmerhackl LB, Felber S, Ischebeck A. (2006). Neural correlates of the number-size interference task in children. *Neuroreport*, 17(6), 587-591.
Kelley WM, Miezin FM, McDermott KB, Buckner RL, Raichle ME, Cohen NJ, Ollinger JM, Akbudak E, Conturo TE, Snyder AZ, Petersen SE. (1998). Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron*, 20(5), 927-936.
Kirchner WK. (1958). Age differences in short-term retention of rapidly changing information. *J Exp Psychol*, 55(4), 352-358.
Klimesch W. (1996). Memory Processes, Brain Oscillations and EEG Synchronization [Review]. *Int J Psychophysiol*, 24(1-2), 61-100.
Klimesch W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Rev*, 29(2-3), 169-195.

Kline CE, Zielinski MR, Devlin TM, Kripke DF, Bogan RK, Youngstedt SD. (2010). Self-reported long sleep in older adults is closely realted to objective time in bed. *Sleep and Biological Rhythms*, 8, 42-51.

Knops A, Thirion B, Hubbard EM, Michel V, Dehaene S. (2009). Recruitment of an area involved in eye movements during mental arithmetic. *Science*, 324(5934), 1583-1585.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A*, 92(19), 8856-8860.

Koten JW, Jr., Wood G, Hagoort P, Goebel R, Propping P, Willmes K, Boomsma DI. (2009). Genetic contribution to variation in cognitive function: an FMRI study in twins. *Science*, 323(5922), 1737-1740.

Kreiner WA. (2003). On the Newcomb-Benford Law. *Zeitschrift für Naturforschung Section A-A Journal of Physical Sciences*, 58(11), 618-622.

Kronholm E, Sallinen M, Suutama T, Sulkava R, Era P, Partonen T. (2009). Self-reported sleep duration and cognitive functioning in the general population. *J Sleep Res*, 18(4), 436-446.

Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. (2008). Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci*, 9(12), 910-919.

Landolt HP. (2008a). Genotype-dependent differences in sleep, vigilance, and response to stimulants. *Curr Pharm Des*, 14(32), 3396-3407.

Landolt HP. (2008b). Sleep homeostasis: A role for adenosine in humans? *Biochem Pharmacol*, 75(11), 2070-2079.

Landolt HP, Dijk DJ, Gaus SE, Borbély AA. (1995). Caffeine reduces low-frequency delta activity in the human sleep EEG. *Neuropsychopharmacology*, 12(3), 229-238.

Landolt HP, Rétey JV, Tönz K, Gottselig JM, Khatami R, Buckelmüller I, Achermann P. (2004). Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. *Neuropsychopharmacology*, 29, 1933-1939.

Landsness EC, Crupi D, Hulse BK, Peterson MJ, Huber R, Ansari H, Coen M, Cirelli C, Benca RM, Ghilardi MF, Tononi G. (2009). Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep*, 32(10), 1273-1284.

Latini S, Pedata F. (2001). Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem*, 79(3), 463-484.

Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. (2008). Self-reported and measured sleep duration: how similar are they? *Epidemiology*, 19(6), 838-845.

Lezak M (1983) Neuropsychological assessment. (Press., N. Y. O. U., ed).

Lim JL, Dinges DF. (2008). Sleep deprivation and vigilant attention. *Ann N Y Acad Sci*, 1129, 305-322.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*, 98(3), 365-376.

Lindskog M, Svenningsson P, Pozzi L, Kim Y, Fienberg AA, Bibb JA, Fredholm BB, Nairn AC, Greengard P, Fisone G. (2002). Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine.[see comment]. *Nature*, 418(6899), 774-778.

Loetscher T, Bockisch CJ, Nicholls ME, Brugger P. (2010). Eye position predicts what number you have in mind. *Curr Biol*, 20(6), R264-265.

Loetscher T, Brugger P. (2007). Exploring number space by random digit generation. *Exp Brain Res*, 180(4), 655-665.

Loetscher T, Brugger P. (2009). Random number generation in neglect patients reveals enhanced response stereotypy, but no neglect in number space. *Neuropsychologia*, 47(1), 276-279.

Loetscher T, Schwarz U, Schubiger M, Brugger P. (2008). Head turns bias the brain's internal random generator. *Curr Biol*, 18(2), R60-62.

Longo MR, Lourenco SF. (2007). Spatial attention and the mental number line: evidence for characteristic biases and compression. *Neuropsychologia*, 45(7), 1400-1407.

Lu B. (2003). Pro-region of neurotrophins: role in synaptic modulation. *Neuron*, 39(5), 735-738.

Lu Y, Christian K, Lu B. (2008). BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiol Learn Mem*, 89(3), 312-323.

Luciano M, Zhu G, Kirk KM, Gordon SD, Heath AC, Montgomery GW, Martin NG. (2007). "No thanks, it keeps me awake": The genetics of coffee-attributed sleep disturbance. *Sleep*, 30(10), 1378-1386.

Luikart BW, Parada. (2006). Receptor tyrosine kinase B-mediated excitatory synaptogenesis *Prog Brain Res*, 157, 15-24.

Luppi PH. (2010). Neurochemical aspects of sleep regulation with specific focus on slow-wave sleep. *World J Biol Psychiatry*, 11 Suppl 1, 4-8.

Mackiewicz M, Nikonova EV, Zimmerman JE, Galante RJ, Zhang L, Cater JR, Geiger JD, Pack AI. (2003). Enzymes of adenosine metabolism in the brain: diurnal rhythm and the effect of sleep deprivation. *J Neurochem*, 85(2), 348-357.

Maquet P. (2001). The role of sleep in learning and memory. *Science*, 294(5544), 1048-1052.

Maret S, Dorsaz S, Gurcel L, Pradervand S, Petit B, Pfister C, Hagenbuchle O, O'Hara BF, Franken P, Tafti M. (2007). Homer1a is a core brain molecular correlate of sleep loss. *Proc Natl Acad Sci U S A*, 104(50), 20090-20095.

Marks GA, Birabil CG. (1998). Enhancement of rapid eye movement sleep in the rat by cholinergic and adenosinergic agonists infused into the pontine reticular formation. *Neuroscience*, 86(1), 29-37.

McCarley RW, Massaquoi SG. (1992). Neurobiological structure of the revised limit cycle reciprocal interaction model of REM cycle control. *J Sleep Res*, 1, 132-137.

McCarthy ME, Waters WF. (1997). Decreased attentional responsivity during sleep deprivation: orienting response latency, amplitude, and habituation. *Sleep*, 20(2), 115-123.

McCormick DA, Pape HC. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. *J Physiol*, 431, 291-318.

McGinty D, Gong H, Suntsova N, Alam MN, Methippara M, Guzman-Marin R, Szymusiak R. (2004). Sleep-promoting functions of the hypothalamic median preoptic nucleus: inhibition of arousal systems. *Arch Ital Biol*, 142(4), 501-509.

McGinty DJ, Harper RM. (1976). Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res*, 101(3), 569-575.

McNair DM, Lorr M, Doppleman LF (1971) Edits Manual for the Profile of Mood States (POMS): Educational and Industrial Testing Service, San Diego, CA.

Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. (2002). The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol*, 59(10), 1553-1562.

Miller GA. (1956). The magical number seven plus or minus two: some limits on our capacity for processing information. *Psychol Rev*, 63(2), 81-97.

Minichiello L. (2009). TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci*, 10(12), 850-860.

Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. (2005). The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. *Am J Med Genet B Neuropsychiatr Genet*, 133B(1), 101-104.

Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. (2004). Behavioral state instability in orexin knock-out mice. *J Neurosci*, 24(28), 6291-6300.

Mograss MA, Guillem F, Stickgold R. (2010). Individual differences in face recognition memory: comparison among habitual short, average, and long sleepers. *Behav Brain Res*, 208(2), 576-583.

Montag C, Weber B, Jentgens E, Elger C, Reuter M. (2010). An epistasis effect of functional variants on the BDNF and DRD2 genes modulates gray matter volume of the anterior cingulate cortex in healthy humans. *Neuropsychologia*, 48(4), 1016-1021.

Monti JM. (1982). Catecholamines and the sleep-wake cycle. I. EEG and behavioral arousal. *Life Sci.*, 30, 1145-1157.

Moore RY, Eichler VB. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res*, 42, 201-206.

Moruzzi G, Magoun HW. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol*, 1(4), 455-473.

Nagappan G, Lu B. (2005). Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends Neurosci*, 28(9), 464-471.

Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 32(4), 392-401.

Nemoto K, Ohnishi T, Mori T, Moriguchi Y, Hashimoto R, Asada T, Kunugi H. (2006). The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neurosci Lett*, 397(1-2), 25-29.

Newcomb S. (1881). Astronomical Observatories. *Science*, 2(60), 377-380.

Nieder A. (2009). Prefrontal cortex and the evolution of symbolic reference. *Curr Opin Neurobiol*, 19(1), 99-108.

Nieder A, Dehaene S. (2009). Representation of number in the brain. *Annu Rev Neurosci*, 32, 185-208.

Ninan I, Bath KG, Dagar K, Perez-Castro R, Plummer MR, Lee FS, Chao MV. (2010). The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *J Neurosci*, 30(26), 8866-8870.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. (2000). Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*, 355(9197), 39-40.
Nixon GM, Thompson JM, Han DY, Becroft DM, Clark PM, Robinson E, Waldie KE, Wild CJ, Black PN, Mitchell EA. (2008). Short sleep duration in middle childhood: risk factors and consequences. *Sleep*, 31(1), 71-78.
Oakley N (1997) Validation with Polysomnography of the Sleepwatch Sleep/Wake Scoring Algorithm used by the Actiwatch Activity Monitoring System: Cambridge Neurotechnology Ltd, Upper Pendrill Court, Ermine St North, Papweorth Everard, Cambridge CB3 8UY, UK.
Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep*, 27(7), 1255-1273.
Okada T, Mochizuki T, Huang ZL, Eguchi N, Sugita Y, Urade Y, Hayaishi O. (2003). Dominant localization of adenosine deaminase in leptomeninges and involvement of the enzyme in sleep. *Biochem Biophys Res Commun*, 312(1), 29-34.
Oken BS, Salinsky MC, Elsas SM. (2006). Vigilance, alertness, or sustained attention: physiological basis and measurement. *Clin Neurophysiol*, 117(9), 1885-1901.
Oliet SH, Mothet JP. (2006). Molecular determinants of D-serine-mediated gliotransmission: from release to function. *Glia*, 54(7), 726-737.
Oliveri M, Rausei V, Koch G, Torriero S, Turriziani P, Caltagirone C. (2004). Overestimation of numerical distances in the left side of space. *Neurology*, 63(11), 2139-2141.
Pace-Schott EF, Hobson JA. (2002). The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nat Rev Neurosci*, 3(8), 591-605.
Palchykova S, Winsky-Sommerer R, Shen HY, Boison D, Gerling A, Tobler I. (2010). Manipulation of adenosine kinase affects sleep regulation in mice. *J Neurosci*, 30(39), 13157-13165.
Perret E. (1974). The left frontal lobe of man and the suppression of habitual responses in verbal categorical behaviour. *Neuropsychologia*, 12(3), 323-330.
Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo MT, Pascucci T, Puglisi-Allegra S, Reichelt KL, Conciatori M, Baldi A, Keller F. (2000). Adenosine deaminase alleles and autistic disorder: case-control and family-based association studies. *Am J Med Genet*, 96(6), 784-790.
Peyron C, Wurts SW, Srere HK, Heller HC, Edgar DM, Kilduff TS. (1998). mRNA level of brain-derived neurotrophic factor increases in several brain regions after sleep deprivation. *Soc Neurosci Abstr*, 24, 1430.
Pinhas M, Fischer MH. (2008). Mental movements without magnitude? A study of spatial biases in symbolic arithmetic. *Cognition*, 109(3), 408-415.
Porkka-Heiskanen T, Strecker RE, McCarley RW. (2000). Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. *Neuroscience*, 99(3), 507-517.
Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW. (1997). Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science*, 276, 1265-1268.
Powell HW, Koepp MJ, Symms MR, Boulby PA, Salek-Haddadi A, Thompson PJ, Duncan JS, Richardson MP. (2005). Material-specific lateralization of memory encoding in the medial temporal lobe: blocked versus event-related design. *Neuroimage*, 27(1), 231-239.
Priftis K, Zorzi M, Meneghello F, Marenzi R, Umilta C. (2006). Explicit versus implicit processing of representational space in neglect: dissociations in accessing the mental number line. *J Cogn Neurosci*, 18(4), 680-688.
Radulovacki M, Virus RM, Djuricic-Nedelson M, Green RD. (1983). Hypnotic Effects of Deoxycorformycin in Rats. *Brain Research*, 271(2), 392-395.
Ravnkilde B, Videbech P, Rosenberg R, Gjedde A, Gade A. (2002). Putative tests of frontal lobe function: a PET-study of brain activation during Stroop's Test and verbal fluency. *J Clin Exp Neuropsychol*, 24(4), 534-547.
Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, Maryland: National Institutes of Health.
Regard M, Strauss E, Knapp P. (1982). Children's production on verbal and non-verbal fluency tasks. *Percept Mot Skills*, 55(3), 839-844.
Regel SJ, Gottselig JM, Schuderer J, Tinguely G, Retey JV, Kuster N, Landolt HP, Achermann P. (2007). Pulsed radio frequency radiation affects cognitive performance and the waking electroencephalogram. *Neuroreport*, 18(8), 803-807.

Rétey JV, Adam M, Honegger E, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P. (2005). A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. *Proc Natl Acad Sci U S A*, 102(43), 15676-15681.

Rétey JV, Adam M, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P. (2007). A Genetic Variation in the Adenosine A_{2A} Receptor Gene (*ADORA2A*) Contributes to Individual Sensitivity to Caffeine Effects on Sleep. *Clin Pharmacol Ther*, 81, 692-698.

Riksen NP, Franke B, van den Broek P, Naber M, Smits P, Rongen GA. (2008). The 22G>A polymorphism in the adenosine deaminase gene impairs catalytic function but does not affect reactive hyperaemia in humans in vivo. *Pharmacogenet Genomics*, 18(10), 843-846.

Roenneberg T, Wirz-Justice A, Merrow M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *J Biol Rhythms*, 18(1), 80-90.

Roffwarg HP, Muzio JN, Dement WC. (1966). Ontogenetic development of the human sleep-dream cycle. *Science*, 152, 604-619.

Rogers L, Andrew R (2002) Comparative vertebrate lateralization. Cambridge, UK.

Ruff RM, Allen CC, Farrow CE, Niemann H, Wylie T. (1994). Figural fluency: differential impairment in patients with left versus right frontal lobe lesions. *Archives of Clinical Neuropsychology*, 9(1), 41-55.

Rusterholz T, Durr R, Achermann P. (2010). Inter-individual differences in the dynamics of sleep homeostasis. *Sleep*, 33(4), 491-498.

Sadeh A, Acebo C. (2002). The role of actigraphy in sleep medicine. *Sleep Med Rev*, 6(2), 113-124.

Sadeh A, Alster J, Urbach D, Lavie P. (1989). Actigraphically based automatic bedtime sleep-wake scoring: Validity and clinical applications. *J Ambulatory Monitoring*, 2, 209-216.

Sadeh A, Hauri PJ, Kripke DF, Lavie P. (1995). The role of actigraphy in the evaluation of sleep disorders. *Sleep*, 18(4), 288-302.

Sagaspe P, Charles A, Taillard J, Bioulac B, Philip P. (2003). [Inhibition and working memory: effect of acute sleep deprivation on a random letter generation task]. *Can J Exp Psychol*, 57(4), 265-273.

Sakurai T. (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci*, 8(3), 171-181.

Sanger F, Nicklen S, Coulson AR. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*, 74(12), 5463-5467.

Saper CB, Chou TC, Scammell TE. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci*, 24(12), 726-731.

Saper CB, Scammell TE, Lu J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063), 1257-1263.

Savitz J, Solms M, Ramesar R. (2006). The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes Brain Behav*, 5(4), 311-328.

Schabus M, Hodlmoser K, Gruber G, Sauter C, Anderer P, Klosch G, Parapatics S, Saletu B, Klimesch W, Zeitlhofer J. (2006). Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. *Eur J Neurosci* 23(7), 1738-1746.

Scharf MT, Naidoo N, Zimmerman JE, Pack AI. (2008). The energy hypothesis of sleep revisited. *Prog Neurobiol*, 86(3), 264-280.

Schmidt C, Collette F, Cajochen C, Peigneux P. (2007). A time to think: Circadian rhythms in human cognition. *Cogn Neuropsychol*, 24(7), 755-789.

Schwierin B, Borbély AA, Tobler I. (1996). Effects of N^6-cyclopentyladenosine and caffeine on sleep regulation in the rat. *Eur J Pharmacol*, 300(3), 163-171.

Sebastiao AM, Ribeiro JA. (2009). Triggering neurotrophic factor actions through adenosine A2A receptor activation: implications for neuroprotection. *Br J Pharmacol*, 158(1), 15-22.

Seugnet L, Boero J, Gottschalk L, Duntley SP, Shaw PJ. (2006). Identification of a biomarker for sleep drive in flies and humans. *Proc Natl Acad Sci U S A*, 103(52), 19913-19918.

Sherin JE, Shiromani PJ, McCarley RW, Saper CB. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science*, 271, 216-219.

Siegel J. (2004a). Brain mechanisms that control sleep and waking. *Naturwiss.*, 91(8), 355-365.

Siegel JM. (2004b). The neurotransmitters of sleep. *J Clin Psychiatry*, 65 Suppl 16, 4-7.

Silva GE, Goodwin JL, Sherrill DL, Arnold JL, Bootzin RR, Smith T, Walsleben JA, Baldwin CM, Quan SF. (2007). Relationship between reported and measured sleep times: the sleep heart health study (SHHS). *J Clin Sleep Med*, 3(6), 622-630.

Sinton CM, McCarley RW. (2004). Neurophysiological mechanisms of sleep and wakefulness: a question of balance. *Semin Neurol*, 24(3), 211-223.

Sivertsen B, Omvik S, Havik OE, Pallesen S, Bjorvatn B, Nielsen GH, Straume S, Nordhus IH. (2006). A comparison of actigraphy and polysomnography in older adults treated for chronic primary insomnia. *Sleep*, 29(10), 1353-1358.

Somers VK, Dyken ME, Mark AL, Abboud FM. (1993). Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med.*, 328, 303-307.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Palo Alto, Calif.: Consulting Psychologists Press.

Stephan FK, Zucker I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci U S A*, 69, 1583-1586.

Steriade M. (2003). The corticothalamic system in sleep. *Front Biosci*, 8, 878-899.

Steriade M, Curró Dossi R, Nuñez A. (1991). Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: Cortically induced synchronization and brainstem cholinergic suppression. *J Neurosci*, 11, 3200-3217.

Steriade M, Jones EG, Llinás RR (1990) Thalamic oscillations and signaling. New York: Wiley.

Steriade M, McCormick DA, Sejnowski TJ. (1993a). Thalamocortical oscillations in the sleeping and aroused brain. *Science*, 262, 679-685.

Steriade M, Nuñez A, Amzica F. (1993b). Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J Neurosci*, 13, 3266-3283.

Steriade M, Nuñez A, Amzica F. (1993c). A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci*, 13, 3252-3265.

Strauss E, Shermann E, Spreen O (2006) A Compendium of Neuropsychological Tests: Oxford University Press, Inc.

Stroop J. (1935). Studies of interference in serial verbal reactions. *J Exp Psychol*, 18(643).

Sturm A, Clarenbach P (1997) Schlafstörungen. Stuttgart, New York: Thieme Verlag.

Sun T, Patoine C, Abu-Khalil A, Visvader J, Sum E, Cherry TJ, Orkin SH, Geschwind DH, Walsh CA. (2005). Early asymmetry of gene transcription in embryonic human left and right cerebral cortex. *Science*, 308(5729), 1794-1798.

Tabor HK, Risch NJ, Myers RM. (2002). Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet*, 3(5), 391-397.

Tafti M. (2009). Genetic aspects of normal and disturbed sleep. *Sleep Med*, 10 Suppl 1, S17-21.

Tang Y, Chorlian DB, Rangaswamy M, O'Connor S, Taylor R, Rohrbaugh J, Porjesz B, Begleiter H. (2007). Heritability of bipolar EEG spectra in a large sib-pair population. *Behav Genet*, 37(2), 302-313.

Tebano MT, Martire A, Rebola N, Pepponi R, Domenici MR, Gro MC, Schwarzschild MA, Chen JF, Cunha RA, Popoli P. (2005). Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: a possible key mechanism in the modulation of N-methyl-D-aspartate effects. *J Neurochem*, 95(4), 1188-1200.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. (2000). Reduced number of hypocretin neurons in human narcolepsy. *Neuron*, 27(3), 469-474.

Thomas M, Sing H, Belenky G, Holcomb H, Mayberg H, Dannals R, Wagner H, Thorne D, Popp K, Rowland L, Welsh A, Balwinski S, Redmond D. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J Sleep Res*, 9(4), 335-352.

Tinguely G, Finelli LA, Landolt HP, Borbely AA, Achermann P. (2006). Functional EEG topography in sleep and waking: state-dependent and state-independent features. *Neuroimage*, 32(1), 283-292.

Tischfield JA, Creagan RP, Nichols EA, Ruddle FH. (1974). Assignment of a gene for adenosine deaminase to human chromosome 20. *Hum Hered*, 24(1), 1-11.

Tononi G, Cirelli C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Res Bull*, 62(2), 143-150.

Tononi G, Cirelli C. (2006). Sleep function and synaptic homeostasis. *Sleep Med Rev*, 10(1), 49-62.

Torsvall L, Åkerstedt T. (1987). Sleepiness on the job: Continuously measured EEG changes in train drivers. *Electroenceph clin Neurophysiol*, 66, 502-511.

Towse JN. (1998). On random generation and the central executive of working memory. *Br J Psychol*, 89 (Pt 1), 77-101.

Towse JN, Neil D. (1998). Analyzing human random generation behavior: A review of methods used and a computer program for describing performance. *Behavior Research Methods Instruments, & Computers*, 30(4), 583-591.

Tucker AM, Dinges DF, Van Dongen HPA. (2007). Trait interindividual differences in the sleep physiology of healthy young adults. *J Sleep Res*, 16, 170-180.

Tunbridge EM, Harrison PJ, Weinberger DR. (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val(158)Met and beyond. *Biol Psychiatry*, 60(2), 141-151.
Turrigiano GG, Nelson SB. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol*, 10(3), 358-364.
Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. (2002). From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem*, 9(5), 224-237.
Urade Y, Eguchi N, Qu W-M, Sakata M, Huang ZL, Chen JF, Schwarzschild MA, Fink JS, Hayaishi O. (2003). Sleep regulation in adenosine A_{2A} receptor-deficient mice. *Neurology*, 61, S94-S96.
Uttl B, Pilkenton-Taylor C. (2001). Letter cancellation performance across the adult life span. *Clin Neuropsychol*, 15(4), 521-530.
van Beijsterveldt CE, Molenaar PC, de Geus EJ, Boomsma DI. (1996). Heritability of human brain functioning as assessed by electroencephalography. *Am J Hum Genet*, 58(3), 562-573.
Van Den Berg JF, Van Rooij FJ, Vos H, Tulen JH, Hofman A, Miedema HM, Neven AK, Tiemeier H. (2008). Disagreement between subjective and actigraphic measures of sleep duration in a population-based study of elderly persons. *J Sleep Res*, 17(3), 295-302.
Van Der Werf YD, Altena E, Schoonheim MM, Sanz-Arigita EJ, Vis JC, De Rijke W, Van Someren EJ. (2009). Sleep benefits subsequent hippocampal functioning. *Nat Neurosci*, 12(2), 122-123.
van Dijck JP, Gevers W, Fias W. (2009). Numbers are associated with different types of spatial information depending on the task. *Cognition*, 113(2), 248-253.
Van Dongen HP, Dinges DF. (2003). Investigating the interaction between the homeostatic and circadian processes of sleep-wake regulation for the prediction of waking neurobehavioural performance. *J Sleep Res*, 12(3), 181-187.
Van Dongen HP, Maislin G, Mullington JM, Dinges DF. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*, 26(2), 117-126.
Van Dongen HP, Vitellaro KM, Dinges DF. (2005). Individual differences in adult human sleep and wakefulness: Leitmotiv for a research agenda. *Sleep*, 28(4), 479-496.
Van Dongen HPA, Baynard MD, Maislin G, Dinges DF. (2004). Systematic interindividual differences in neurobehavioral impairment from sleep loss: Evidence of trait-like differential vulnerability. *Sleep*, 27(3), 423-433.
van Stegeren A, Rohleder N, Everaerd W, Wolf OT. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*, 31(1), 137-141.
Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M. (2002). Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Mol Psychiatry*, 7(2), 136-137.
Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ. (2007). PER3 polymorphism predicts sleep structure and waking performance. *Current Biology*, 17(7), 613-618.
Von Economo C. (1930). Sleep as a problem of localization. *J Nerv Ment Dis*, 71, 249-259.
von Zerssen D, Doerr P, Emrich HM, Lund R, Pirke KM. (1987). Diurnal variation of mood and the cortisol rhythm in depression and normal states of mind. *Eur Arch Psychiatr Neurol Sci*, 237, 36-45.
Walker MP, Stickgold R. (2006). Sleep, memory, and plasticity. *Annu Rev Psychol*, 57, 139-166.
Waterhouse EG, Xu B. (2009). New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. *Mol Cell Neurosci*, 42(2), 81-89.
Watson NF, Kapur V, Arguelles LM, Goldberg J, Schmidt DF, Armitage R, Buchwald D. (2003). Comparison of subjective and objective measures of insomnia in monozygotic twins discordant for chronic fatigue syndrome. *Sleep*, 26(3), 324-328.
Webb WB. (1979). Are short and long sleepers different? *Psychol Rep*, 44, 259-264.
Weintraub S, Mesulam MM. (1988). Visual hemispatial inattention: stimulus parameters and exploratory strategies. *J Neurol Neurosurg Psychiatry*, 51(12), 1481-1488.
Wisor JP, Eriksson KS. (2005). Dopaminergic-adrenergic interactions in the wake promoting mechanism of modafinil. *Neuroscience*, 132(4), 1027-1034.
Wood G, Fischer MH. (2008). Numbers, space, and action - from finger counting to the mental number line and beyond. *Cortex*, 44(4), 353-358.
Wood G, Nuerk HC, Willmes K. (2006). Crossed hands and the SNARC effect: a failure to replicate Dehaene, Bossini and Giraux (1993). *Cortex*, 42(8), 1069-1079; discussion 1119-1023.

Wood G, Nuerk HC, Willmes K, Fischer MH. (2008). On the cognitive link between space and number: A meta-analysis of the SNARC effect. *Psychol Sci Q*, 50(4), 489-525.

Yaar R, Jones MR, Chen JF, Ravid K. (2005). Animal models for the study of adenosine receptor function. *J Cell Physiol*, 202(1), 9-20.

Yamada K, Nabeshima T. (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci*, 91(4), 267-270.

Yovel G, Tambini A, Brandman T. (2008). The asymmetry of the fusiform face area is a stable individual characteristic that underlies the left-visual-field superiority for faces. *Neuropsychologia*, 46(13), 3061-3068.

Zorzi M, Priftis K, Meneghello F, Marenzi R, Umilta C. (2006). The spatial representation of numerical and non-numerical sequences: evidence from neglect. *Neuropsychologia*, 44(7), 1061-1067.

Zorzi M, Priftis K, Umilta C. (2002). Brain damage: neglect disrupts the mental number line. *Nature*, 417(6885), 138-139.

Zuccato C, Cattaneo E. (2009). Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat Rev Neurol*, 5(6), 311-322.

Appendix I

Supplementary information to chapter 5

The functional p.Asp8Asn polymorphism of adenosine deaminase (ADA) predicts inter-Individual differences in homeostatic sleep pressure

Table S1. Cognitive assessment of study participants					
	Asp/Asn	Asp/Asp	*'genotype'*	*'gender'*	*'genotype*gender'*
Assessment of attention					
d2 Task:			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Number of processed items	503.2 ± 12.4	533.7 ± 5.3	4.6 (0.03)	1.0 (0.31)	0.3 (0.60)
Sum of omission and commission errors	21.4 ± 3.3	24.0 ± 1.4	0.6 (0.45)	0.7 (0.40)	2.0 (0.16)
Total number of items processed minus errors	481.8 ± 12.5	509.6 ± 5.5	3.6 (0.06)	1.4 (0.23)	0.0 (0.87)
Fluctuation rate	11.4 ± 0.7	11.5 ± 0.3	0.0 (0.93)	0.1 (0.78)	0.2 (0.66)
Assessment of memory: Learning efficiency and memory decline					
Rey Auditory Verbal Learning Test (RAVLT)			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Total number of total words recalled across five trials	56.7 ± 1.5	55.5 ± 0.6	0.6 (0.45)	3.6 (0.06)	0.0 (0.93)
Immediate recall	12.6 ± 0.3	12.5 ± 0.1	0.1 (0.78)	6.8 (0.01)	0.2 (0.68)
Delayed recall	12.7 ± 0.4	12.3 ± 0.2	1.1 (0.29)	12.3 (0.001)	0.9 (0.34)
Rey Visual Design Learning Test (RVDLT)			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Total number of total figures recalled across five trials	55.7 ± 2.0	55.5 ± 0.6	0.2 (0.64)	1.6 (0.20)	1.2 (0.27)
Immediate recall	13.1 ± 0.5	13.1 ± 0.2	0.0 (0.86)	1.9 (0.17)	1.5 (0.22)
Delayed recall	13.1 ± 0.5	13.2 ± 0.2	0.0 (0.93)	1.7 (0.19)	1.3 (0.26)
Assessment of memory: Working memory					
Digit span test			$F_{1, 215}$ (p)	$F_{1, 215}$ (p)	$F_{1, 215}$ (p)
Number of digits recalled during forward run	6.6 ± 0.2	6.8 ± 0.1	0.8 (0.37)	0.7 (0.40)	0.2 (0.70)
Number of digits recalled during backward run	5.6 ± 0.3	5.6 ± 0.1	0.0 (0.91)	1.4 (0.24)	7.6 (0.01)

Assessment of executive functions					
Stroop color-word test			**$F_{1, 216}$ (p)**	**$F_{1, 216}$ (p)**	**$F_{1, 216}$ (p)**
Interference score in milliseconds (ms)	6.4 ± 0.6	7.0 ± 0.3	0.5 (0.49)	1.6 (0.21)	0.1 (0.76)
Random Number Generation Test			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Redundancy	0.9 ± 0.1	0.8 ± 0.0	0.5 (0.50)	0.4 (0.54)	0.1 (0.74)
Adjacency	35.0 ± 1.2	38.1 ± 0.7	3.1 (0.08)	0.0 (0.99)	2.5 (0.11)
Go/ no-go Task			$F_{1, 209}$ (p)	$F_{1, 209}$ (p)	$F_{1, 209}$ (p)
Reaction time in milliseconds (ms)	429.0 ± 6.2	418.2 ± 3.3	1.5 (0.23)	0.6 (0.45)	1.7 (0.19)
Nonverbal fluency test (5 point test)			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Sum of admissible figures designed during 3 minutes	44.7 ± 1.9	45.2 ± 0.7	0.1 (0.83)	0.1 (0.72)	0.1 (0.70)
Verbal fluency test (s word test)			$F_{1, 216}$ (p)	$F_{1, 216}$ (p)	$F_{1, 216}$ (p)
Sum of admissible words produced during 3 minutes	34.0 ± 1.7	35.6 ± 0.7	0.7 (0.42)	6.5 (0.01)	3.6 (0.06)

Table S2. Subject Characteristics

	p.Asp8Asn genotype		*p*-value
	Asp/Asn	**Asp/Asp**	
Sex (females / males)	5/6	5/6	n/a
Age (years)	24.3 ± 1.2	24.5 ± 1.0	0.7
Years of education	12.1 ± 0.5	12.7 ± 0.5	0.4
Alcohol consumption (drinks/week)	4.0 ± 0.9	2.6 ± 0.6	0.2
Caffeine consumption (mg/day)	149.6 ± 33.6	181.4 ± 38.4	0.6
Body mass index (kg/m^2)	21.8 ± 0.6	21.9 ± 0.4	0.9
Trait Anxiety	33.0 ± 1.9	35.5 ± 2.4	0.5
Diurnal preference	2.6 ± 0.2	3.0 ± 0.2	0.1
Daytime sleepiness	6.5 ± 1.0	5.0 ± 0.8	0.3

Values represent means ± SEM (n = 11 per group).
Values of caffeine consumption were based on the following average caffeine content per serving: Coffee: 100 mg; Ceylon or green tea: 30 mg; Cola drink: 40 mg (2 dl); Energy drink: 80 mg (2 dl); Chocolate: 50 mg (100 g). Diurnal preference ("Chronotype"), trait anxiety and daytime sleepiness were assessed with the Horne-Östberg Morningness-Eveningness Questionnaire, the Trait-State Anxiety Inventory (Spielberger et al., 1970) and the Epworth Sleepiness Scale (Johns, 1991), respectively. *P*-values refer to two-tailed, paired *t*-tests.

Table S3. The p.Asp/Asn polymorphism of *ADA* does not affect habitual sleep duration

Sleep duration	Asp/Asn genotype		Asp/Asp genotype	
	MCTQ	Actigraphy	MCTQ	Actigraphy
Work days	7.2 ± 0.4	7.3 ± 0.2	7.3 ± 0.3	7.5 ± 0.2
Leisure days	8.6 ± 0.4	8.2 ± 0.3	8.2 ± 0.3	8.2 ± 0.4
Average (work and leisure days)	7.6 ± 0.3	7.6 ± 0.2	7.6 ± 0.3	7.7 ± 0.2

Mean values ± SEM (hours) in 11 Asp/Asn and 11 Asp/Asp allele carriers. Average values over work and leisure days were calculated by weighing 5 work and 2 leisure days. MCTQ: Munich Chronotype Questionnaire (Roenneberg et al., 2003). To objectively estimate habitual sleep duration, volunteers continuously wore a rest-activity monitor (actigraphy) and kept a sleep-wake diary during 4 weeks at home. The monitor data were missing in 2 Asp/Asp genotype subjects. Sleep duration refers to the difference between start and end times of the estimated nocturnal rest episode. In both genotypes, MCTQ and actigraphy revealed longer sleep on leisure days than on work days ($p_{all} < 0.006$, two-tailed, paired *t*-tests).

Table S4. Visually scored sleep variables in baseline and recovery nights.

Variable	**Asp/Asn genotype**		**Asp/Asp genotype**		*'genotype'*	*'condition'*	*'genotype' x 'condition'*
	Baseline	Recovery	Baseline	Recovery	$F_{1,30}$ (p <)	$F_{1,30}$ (p <)	$F_{1,30}$ (p <)
Sleep episode	466.1 ± 4.4	476.2 ± 0.8	463.0 ± 4.3	476.9 ± 0.5	0.2 (0.70)	14.7 (0.001)	0.4 (0.55)
Total sleep time	453.6 ± 5.7	466.7 ± 1.3	447.7 ± 4.4	465.9 ± 1.2	0.8 (0.37)	18.8 (0.001)	0.5 (0.49)
Sleep efficiency	94.6 ± 1.2	97.2 ± 0.3	93.4 ± 0.9	97.1 ± 0.2	0.9 (0.36)	18.0 (0.001)	0.5 (0.48)
Sleep latency	13.3 ± 4.4	3.8 ± 0.8	16.5 ± 4.3	3.2 ± 0.5	0.2 (0.69)	13.6 (0.001)	0.4 (0.55)
REM latency	76.1 ± 8.9	91.2 ± 13.1	73.9 ± 7.3	73.2 ± 8.8	1.1 (0.31)	0.5 (0.47)	0.7 (0.43)
WASO	3.9 ± 1.6	0.5 ± 0.2	4.2 ± 0.6	1.0 ± 0.3	0.1 (0.72)	8.6 (0.01)	0.01 (0.94)
Stage 1	32.2 ± 3.9	20.1 ± 5.3	31.7 ± 3.9	16.2 ± 3.2	0.4 (0.52)	17.6 (0.001)	0.3 (0.61)
Stage 2	196.6 ± 7.9	162.7 ± 4.5**	212.1 ± 4.8	207.1 ± 6.2	26.7 (0.001)	11.3 (0.003)	6.2 (0.02)
Slow wave sleep	123.9 ± 7.2**	191.2 ± 8.2**	100.3 ± 6.1	153.0 ± 4.2	51.6 (0.001)	194.7 (0.001)	2.9 (0.11)
REM sleep	100.9 ± 5.4	92.8 ± 3.8	103.6 ± 4.2	89.7 ± 7.0	0 (0.98)	4.5 (0.05)	0.3 (0.59)
Movement time	8.7 ± 0.8	9.0 ± 0.9	11.1 ± 1.7	9.9 ± 1.1	3.0 (0.10)	0.2 (0.68)	0.7 (0.43)

Mean values ± SEM in 11 Asp/Asn and 11 Asp/Asp allele carriers in minutes (except sleep efficiency [%]) for the first 480 minutes from lights-off. Baseline: baseline night. Recovery: recovery night after 40 hours prolonged wakefulness. Sleep episode: time after sleep onset until final awakening. Sleep efficiency: total sleep time per 480 min. Sleep latency: time from lights-off to first occurrence of stage 2 sleep. REM latency: time from sleep onset to first occurrence of REM sleep. WASO: waking after sleep onset. Slow wave sleep: combined stages 3 & 4.

F- and *p*-values: 2-way mixed-model ANOVA with within-subjects factors 'genotype' (Asp/Asn, Asp/Asp) and 'condition' (baseline, recovery).

****** $p < 0.001$ (Asp/Asn *vs.* Asp/Asp genotype; two-tailed, paired *t*-test)

Curriculum vitae

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List of publications

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Bachmann V, Muller R, vanHedel HJ, Dietz, V (2008) Vertical perturbations of human gait: organization and adaptation of leg muscle responses. Exp Brain Res 186(1): 123-30.

Oral presentations at national and international meetings

20th Congress of the European Sleep Research Society (ESRS), September 14 -18, 2010, Lisbon, Portugal

„Effects of 22G>A polymorphism of adenosine deaminase on waking performance and response to sleep deprivation in humans"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, April 15 2010

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Kolloquium für Klinische und Experimentelle Neuropsychologie (Klinex), Wednesday, March 24, 2010, Departement of Neurology, Zürich

"Functional genetic variation in adenosine deaminase, attention and sleep-wake regulation in humans"

Nacht der Forschung, Friday, September 25, 2009, Zürich

"Der Massstab im Kopf"

5th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP), Friday, August 28, 2009, Zürich

"Genetic variation in adenosine deaminase, attention and sleep in humans"

Progress Report Seminar, Institute of Pharmacology and Toxicology, University of Zürich, Zürich, July 2, 2009

„Functional genetic variation of adenosine deaminase, sleep and performance"

4th ZIHP Retreat, PhD Program, June 10-11, 2009, Oberschan

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Poster presentations at national and international meetings

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ZNZ Symposium 2009, Friday, September 11, 2009, Zürich Hönggerberg

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Bachmann V, Klaus F, Bodenmann S, Schäfer N, Brugger P, Landolt HP "A functional genetic variation of adenosine deaminase modulates waking performance irrespective of self-rated sleep duration"

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4th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP), Friday, August 22, 2008, Zürich

Bachmann V, Nalkara P, Tutal M, Landolt HP, Brugger B "Asymmetric prefrontal cortex functions predict asymmetries in number space in healthy adults"

4th Symposium of the Zurich Center for Integrative Human Physiology (ZIHP), Friday, August 22, 2008, Zürich

Bachmann V, Brugger P, Landolt HP "Relationship between distinct cognitive abilities and self-estimated sleep duration in healthy adults"

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Bachmann V, Brugger P, Landolt HP "Relationship between distinct cognitive abilities and self-estimated sleep duration in healthy adults"

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Bachmann V, Brugger P, Landolt HP "Relationship between distinct cognitive abilities and self-estimated sleep duration in healthy adults"

Pharmacology Posterday 2008, Tuesday, Mai 27, 2008, Irchel, Zürich

Bachmann V, Brugger P, Landolt HP "Relationship between distinct cognitive abilities and self-estimated sleep duration in healthy adults"

References

Acebo C, LeBourgeois MK. (2006). Actigraphy. *Respir Care Clin N Am*, 12(1), 23-30.

Achermann P. (2004). The two-process model of sleep regulation revisited. *Aviat Space Environ Med*, 75(3 Suppl), A37-43.

Achermann P, Borbély AA. (1997). Low-frequency (< 1 Hz) oscillations in the human sleep EEG. *Neuroscience*, 81, 213-222.

Adam M, Rétey JV, Khatami R, Landolt HP. (2006). Age-related changes in the time course of vigilant attention during 40 hours without sleep in men. *Sleep*, 29, 55-57.

Adamantidis AR, Zhang F, Aravanis AM, Deisseroth K, de Lecea L. (2007). Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature*, 450(7168), 420-424.

Aeschbach D, Cajochen C, Landolt HP, Borbély AA. (1996). Homeostatic sleep regulation in habitual short sleepers and long sleepers. *Am J Physiol*, 270(1), R41-R53.

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, Giesen HA, Wehr TA. (2000). Short sleepers tolerate a higher homeostatic sleep pressure than long sleepers: evidence from the waking electroencephalogram. *Sleep*, 23, A34.

Aeschbach D, Postolache TT, Sher L, Matthews JR, Jackson MA, Wehr TA. (2001). Evidence from the waking electroencephalogram that short sleepers live under higher homeostatic sleep pressure than long sleepers. *Neuroscience*, 102(3), 493-502.

Aguilar-Roblero R, Verduzco-Carbajal L, Rodriguez C, Mendez-Franco J, Moran J, Perez de la Mora M. (1993). Circadian rhythmicity in the GABAergic system in the suprachiasmatic nuclei of the rat. *Neuroscience Letters*, 157, 199-202.

Akerstedt T, Gillberg M. (1990). Subjective and objective sleepiness in the active individual. *Int J Neurosci*, 52(1-2), 29-37.

Alhaider IA, Aleisa AM, Tran TT, Alkadhi KA. (2010). Caffeine prevents sleep loss-induced deficits in long-term potentiation and related signaling molecules in the dentate gyrus. *Eur J Neurosci*, 31(8), 1368-1376.

Allebrandt KV, Roenneberg T. (2008). The search for circadian clock components in humans: new perspectives for association studies. *Braz J Med Biol Res*, 41(8), 716-721.

Allebrandt KV, Teder-Laving M, Akyol M, Pichler I, Muller-Myhsok B, Pramstaller P, Merrow M, Meitinger T, Metspalu A, Roenneberg T. (2010). CLOCK gene variants associate with sleep duration in two independent populations. *Biol Psychiatry*, 67(11), 1040-1047.

Ambrosius U, Lietzenmaier S, Wehrle R, Wichniak A, Kalus S, Winkelmann J, Bettecken T, Holsboer F, Yassouridis A, Friess E. (2008). Heritability of sleep electroencephalogram. *Biol Psychiatry*, 64, 344-348.

Amzica F, Steriade M. (1997). The K-complex - its slow (<1-Hz) rhythmicity and relation to delta waves. *Neurology*, 49(4), 952-959.

Amzica F, Steriade M. (2002). The functional significance of K-complexes. *Sleep Med Rev*, 6(2), 139-149.

Ancoli-Israel S, Cole R, Alessi C, Chambers M, Moorcroft W, Pollak CP. (2003). The role of actigraphy in the study of sleep and circadian rhythms. *Sleep*, 26(3), 342-392.

Andretic R, Franken P, Tafti M. (2008). Genetics of sleep. *Annu Rev Genet*, 42, 361-388.

Archer SN, Robilliard DL, Skene DJ, Smits M, Williams A, Arendt J, von Schantz M. (2003). A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep*, 26(4), 413-415.

Aserinsky E, Kleitman N. (1953). Regularly occurring periods of eye motility, and concomitant phenomena, during sleep. *Science*, 118, 273-274.

Assaife-Lopes N, Sousa VC, Pereira DB, Ribeiro JA, Chao MV, Sebastiao AM. (2010). Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF-mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. *J Neurosci*, 30(25), 8468-8480.

Aston-Jones G, Bloom FE. (1981). Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci*, 1(8), 876-886.

Bachmann V, Klaus F, Bodenmann S, Schäfer N, Brugger P, Huber S, Berger W, Landolt HP. (2011). The functional Asp8Asn polymorphism of adenosine deaminase (ADA) predicts inter-indivdual differences in homeostatic sleep pressure. (submitted).

Backhaus J, Hoeckesfeld R, Born J, Hohagen F, Junghanns K. (2008). Immediate as well as delayed post learning sleep but not wakefulness enhances declarative memory consolidation in children. *Neurobiol Learn Mem*, 89(1), 76-80.

Baddeley A, Emslie H, Kolodny J, Duncan J. (1998). Random generation and the executive control of working memory. *Q J Exp Psychol A*, 51(4), 819-852.
Baldo JV, Shimamura AP, Delis DC, Kramer J, Kaplan E. (2001). Verbal and design fluency in patients with frontal lobe lesions. *J. Int. Neuropsychol. Soc.*, 7(5), 586-596.
Barker PA. (2009). Whither proBDNF? *Nat Neurosci*, 12(2), 105-106.
Basheer R, Bauer A, Elmenhorst D, Ramesh V, McCarley RW. (2007). Sleep deprivation upregulates A1 adenosine receptors in the rat basal forebrain. *Neuroreport*, 18(18), 1895-1899.
Basheer R, Strecker RE, Thakkar MM, McCarley RW. (2004). Adenosine and sleep-wake regulation. *Prog Neurobiol*, 73, 379-396.
Bath KG, Lee FS. (2006). Variant BDNF (Val66Met) impact on brain structure and function. *Cogn Affect Behav Neurosci*, 6(1), 79-85.
Battistuzzi G, Iudicone P, Santolamazza P, Petrucci R. (1981). Activity of adenosine deaminase allelic forms in intact erythrocytes and in lymphocytes. *Ann Hum Genet*, 45(Pt 1), 15-19.
Bauer A, Ishiwata K. (2009). Adenosine receptor ligands and PET imaging of the CNS. *Handb Exp Pharmacol*(193), 617-642.
Benford F. (1938). The law of anomalous numbers. *Proceedings of the American Philosophical Society*, 78(4), 551-572.
Benington JH, Heller HC. (1995). Restoration of brain energy metabolism as the function of sleep. *Prog Neurobiol*, 45, 347-360.
Berger H. (1929). Über das Elektroenzephalogramm des Menschen. *Arch. Psychiat. Nervenkr.*, 87, 527-570.
Binder DK, Scharfman HE. (2004). Brain-derived neurotrophic factor. *Growth Factors*, 22(3), 123-131.
Binks PG, Waters WF, Hurry M. (1999). Short-term total sleep deprivations does not selectively impair higher cortical functioning. *Sleep*, 22(3), 328-334.
Bjorness TE, Kelly CL, Gao T, Poffenberger V, Greene RW. (2009). Control and function of the homeostatic sleep response by adenosine A1 receptors. *J Neurosci*, 29(5), 1267-1276.
Bliss TV, Lomo T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol*, 232(2), 331-356.
Bodenmann S. (2009). Sleep-wake regulation in humans: pharmacogenetic studies. *Dissertation*.
Bodenmann S, Rusterholz T, Durr R, Stoll C, Bachmann V, Geissler E, Jaggi-Schwarz K, Landolt HP. (2009a). The functional Val158Met polymorphism of COMT predicts interindividual differences in brain alpha oscillations in young men. *J Neurosci*, 29(35), 10855-10862.
Bodenmann S, Xu S, Luhmann UF, Arand M, Berger W, Jung HH, Landolt HP. (2009b). Pharmacogenetics of modafinil after sleep loss: catechol-O-methyltransferase genotype modulates waking functions but not recovery sleep. *Clin Pharmacol Ther*, 85(3), 296-304.
Bodizs R, Kis T, Lazar AS, Havran L, Rigo P, Clemens Z, Halasz P. (2005). Prediction of general mental ability based on neural oscillation measures of sleep. *J Sleep Res*, 14(3), 285-292.
Bongard S, Nieder A. (2010). Basic mathematical rules are encoded by primate prefrontal cortex neurons. *Proc Natl Acad Sci U S A*, 107(5), 2277-2282.
Borbély, Acherman P (2000) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine(Kryger, M. H. et al., eds), pp 377-390 Philadelphia, PA: W.B. Saunders Company.
Borbely AA. (1982). A two process model of sleep regulation. *Hum Neurobiol*, 1(3), 195-204.
Borbély AA. (1984). Schlafgewohnheiten,Schlafqualität und Schlafmittelkonsum der Schweizer Bevölkerung. Ergebnisse einer Repräsentativumfrage. *Schweizerische Ärztezeitung*, 65, 1606-1613.
Borbély AA, Achermann P (2005) Sleep homeostasis and models of sleep regulation. In: Principles and Practice of Sleep Medicine(Kryger, M. H. et al., eds), pp 405-417 Philadelphia, PA: Elsevier Saunders.
Borroni AM, Fichtenholtz H, Woodside BL, Teyler TJ. (2000). Role of voltage-dependent calcium channel long-term potentiation (LTP) and NMDA LTP in spatial memory. *J Neurosci*, 20(24), 9272-9276.
Bottini G, Toraldo A. (2003). The influence of contralesional targets on the cancellation of ipsilesional targets in unilateral neglect. *Brain Cogn*, 53(2), 117-120.
Bowers D, Heilman KM. (1980). Pseudoneglect: effects of hemispace on a tactile line bisection task. *Neuropsychologia*, 18(4-5), 491-498.
Bradshaw JL, Nathan G, Nettleton NC, Wilson L, Pierson J. (1987). Why is there a left side underestimation in rod bisection? *Neuropsychologia*, 25(4), 735-738.
Bradshaw JL, Nettleton NC, Nathan G, Wilson L. (1983). Head and body space to left and right, front and rear--II. Visuotactual and kinesthetic studies and left-side underestimation. *Neuropsychologia*, 21(5), 475-486.
Bremer F. (1935). Cereveau isole et physiologie du sommeil. *CR Soc Biol (Paris)*, 118, 1235-1242.

Brickenkamp R. (1962). Aufmerksamkeits-Belastungs-Test (Test d2). [The d2 test of Attention]. *Göttingen:Hogrefe*.

Brugger P, Monsch AU, Johnson SA. (1996a). Repetitive behavior and repetition avoidance: the role of the right hemisphere. *J Psychiatry Neurosc.*, 21(1), 53-56.

Brugger P, Monsch AU, Salmon DP, Butters N. (1996b). Random number generation in dementia of the Alzheimer type: a test of frontal executive functions. *Neuropsychologia*, 34(2), 97-103.

Brugger P, Schubiger M, Loetscher T. (2010). Leftward bias in number space is modulated by magical ideation. *Cogn Behav Neurol*, 23(2), 119-123.

Brunoni AR, Lopes M, Fregni F. (2008). A systematic review and meta-analysis of clinical studies on major depression and BDNF levels: implications for the role of neuroplasticity in depression. *Int J Neuropsychopharmacol*, 11(8), 1169-1180.

Buchmann A, Ringli M, Kurth S, Schaerer M, Geiger A, Jenni OG, Huber R. (2010). EEG Sleep Slow-Wave Activity as a Mirror of Cortical Maturation. *Cereb Cortex*.

Buckelmüller J, Landolt HP, Stassen HH, Achermann P. (2006). Trait-like individual differences in the human sleep electroencephalogram. *Neuroscience*, 138(1), 351-356.

Burns B. (2009). Sensitivity to statistical regularities: People (largely) follow Benford's law. *Proceedings of the Cognitive Science Societey*, 637.

Cajochen C, Brunner DP, Kräuchi K, Graw P, Wirz-Justice A. (1995). Power density in theta/alpha frequencies of the waking EEG progressively increases during sustained wakefulness. *Sleep*, 18(10), 890-894.

Cajochen C, Foy R, Dijk DJ. (1999). Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Sleep Research Online*, 2(3), 65-69.

Cajochen C, Knoblauch V, Kräuchi K, Renz C, Wirz-Justice A. (2001). Dynamics of frontal EEG activity, sleepiness and body temperature under high and low sleep pressure. *Neuroreport*, 12(10), 2277-2281.

Cajochen C, Wyatt JK, Czeisler CA, Dijk DJ. (2002). Separation of circadian and wake duration-dependent modulation of EEG activation during wakefulness. *Neuroscience*, 114(4), 1047-1060.

Cantlon JF, Libertus ME, Pinel P, Dehaene S, Brannon EM, Pelphrey KA. (2009). The neural development of an abstract concept of number. *J Cogn Neurosci*, 21(11), 2217-2229.

Carvalho AL, Caldeira MV, Santos SD, Duarte CB. (2008). Role of the brain-derived neurotrophic factor at glutamatergic synapses. *Br J Pharmacol*, 153 Suppl 1, 310-324.

Casasanto D. (2009). Embodiment of abstract concepts: good and bad in right- and left-handers. *J Exp Psychol Gen*, 138(3), 351-367.

Chamberlin NL, Arrigoni E, Chou TC, Scammell TE, Greene RW, Saper CB. (2003). Effects of adenosine on GABAergic synaptic inputs to identified ventrolateral preoptic neurons. *Neuroscience*, 119(4), 913-918.

Chapman LJ, Chapman JP. (1987). The measurement of handedness. *Brain Cogn*, 6(2), 175-183.

Chee MW, Choo WC. (2004). Functional imaging of working memory after 24 hr of total sleep deprivation. *J Neurosci*, 24(19), 4560-4567.

Chen ZY, Jing D, Bath KG, Ieraci A, Khan T, Siao CJ, Herrera DG, Toth M, Yang C, McEwen BS, Hempstead BL, Lee FS. (2006). Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science*, 314(5796), 140-143.

Chen ZY, Patel PD, Sant G, Meng CX, Teng KK, Hempstead BL, Lee FS. (2004). Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci*, 24(18), 4401-4411.

Cirelli C. (2002). How sleep deprivation affects gene expression in the brain: a review of recent findings. *J Appl Physiol*, 92(1), 394-400.

Cirelli C, Tononi G. (2000). Differential expression of plasticity-related genes in waking and sleep and their regulation by the noradrenergic system. *J Neurosci*, 20(24), 9187-9194.

Contreras D, Steriade M. (1995). Cellular basis of EEG slow rhythms: a study of dynamic corticothalamic relationships. *J Neurosci*, 15, 604-622.

Contreras D, Steriade M. (1996). Spindle oscillation in cats: the role of corticothalamic feedback in a thalamically generated rhythm. *J Physiol*, 490, 159-179.

Cooley WJ, Tukey JW. (1965). An algorithm for the machine calculation of complex Fourier series. *Math Comput*, 19, 297-301.

Crocker A, Sehgal A. (2010). Genetic analysis of sleep. *Genes Dev*, 24(12), 1220-1235.

Cunha C, Brambilla R, Thomas KL. (2010). A simple role for BDNF in learning and memory? *Front Mol Neurosci*, 3, 1.

Daniels C, Witt K, Wolff S, Jansen O, Deuschl G. (2003). Rate dependency of the human cortical network subserving executive functions during generation of random number series--a functional magnetic resonance imaging study. *Neurosci Lett*, 345(1), 25-28.

de Frias CM, Annerbrink K, Westberg L, Eriksson E, Adolfsson R, Nilsson LG. (2004). COMT gene polymorphism is associated with declarative memory in adulthood and old age. *Behav Genet*, 34(5), 533-539.

De Gennaro L, Marzano C, Fratello F, Moroni F, Pellicciari MC, Ferlazzo F, Costa S, Couyoumdjian A, Curcio G, Sforza E, Malafosse A, Finelli L, Pasqualetti P, Ferrara M, Bertini M, Rossini PM. (2008). The EEG fingerprint of sleep is genetically determined: a twin study. *Ann Neurol*, 64(4), 455-460.

Dehaene S (1997) The number sense. How the mind creates mathematics: Oxford: Oxford University Press.

Dehaene S, Bossini S, Giraux P. (1993). The mental representation of parity and number magnitude. *J Exp Psychol Gen*, 122, 371-396.

Dehaene S, Mehler J. (1992). Cross-linguistic regularities in the frequency of number words. *Cognition*, 43(1), 1-29.

Dement W, Kleitman N. (1957). Cyclic variations in EEG during sleep and their relation to eye movements, body motility, and dreaming. *EEG Clin Neurophysiol*, 9, 673-690.

Dempster E, Toulopoulou T, McDonald C, Bramon E, Walshe M, Filbey F, Wickham H, Sham PC, Murray RM, Collier DA. (2005). Association between BDNF Val(66)Met genotype and episodic memory. *Am J Med Genet B Neuropsychiatr Genet*, 134B(1), 73-75.

Diekamp B, Regolin L, Gunturkun O, Vallortigara G. (2005). A left-sided visuospatial bias in birds. *Curr Biol*, 15(10), 372-373.

Diekmann T. (2007). Not the first digit! Using Benford's Law to detect fraudulent scientific data. *J Appl Stat*, 34(3), 321-329.

Dijk DJ, Archer SN. (2010). PERIOD3, circadian phenotypes, and sleep homeostasis. *Sleep Med Rev*, 14(3), 151-160.

Dijk DJ, Czeisler CA. (1994). Paradoxical Timing of the Circadian-Rhythm of Sleep Propensity Serves to Consolidate Sleep and Wakefulness in Humans. *Neuroscience Letters*, 166(1), 63-68.

Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, Aptowicz C, Pack AI. (1997). Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep*, 20(4), 267-277.

Doricchi F, Guariglia P, Gasparini M, Tomaiuolo F. (2005). Dissociation between physical and mental number line bisection in right hemisphere brain damage. *Nat Neurosci*, 8(12), 1663-1665.

Doricchi F, Merola S, Aiello M, Guariglia P, Bruschini M, Gevers W, Gasparini M, Tomaiuolo F. (2009). Spatial orienting biases in the decimal numeral system. *Curr Biol*, 19(8), 682-687.

Dorrian J, Rogers NL, Dinges DF (2005) Psychomotor vigilance performance: a neurocognitive assay sensitive to sleep loss. In: Sleep Deprivation: Clinical Issues, Pharmacology and Sleep Loss Effects (Kushida, C., ed), pp 39-70 New York: Marcel Dekker, Inc.

Drummond SP, Bischoff-Grethe A, Dinges DF, Ayalon L, Mednick SC, Meloy MJ. (2005). The neural basis of the psychomotor vigilance task. *Sleep*, 28(9), 1059-1068.

Drummond SP, Brown GG, Gillin JC, Stricker JL, Wong EC, Buxton RB. (2000). Altered brain response to verbal learning following sleep deprivation. *Nature*, 403(6770), 655-657.

Drummond SP, Brown GG, Stricker JL, Buxton RB, Wong EC, Gillin JC. (1999). Sleep deprivation-induced reduction in cortical functional response to serial subtraction. *Neuroreport*, 10(18), 3745-3748.

Duncan J, Seitz RJ, Kolodny J, Bor D, Herzog H, Ahmed A, Newell FN, Emslie H. (2000). A neural basis for general intelligence. *Science*, 289(5478), 457-460.

Durmer JS, Dinges DF. (2005). Neurocognitive consequences of sleep deprivation. *Semin Neurol*, 25(1), 117-129.

Ebersbach G, Trottenberg T, Hattig H, Schelosky L, Schrag A, Poewe W. (1996). Directional bias of initial visual exploration. A symptom of neglect in Parkinson's disease. *Brain*, 119 (Pt 1), 79-87.

Egan MF, Goldberg TE, Kolachana BS, Callicott JH, Mazzanti CM, Straub RE, Goldman D, Weinberger DR. (2001). Effect of COMT Val(108/158) Met genotype on frontal lobe function and risk for schizophrenia. *Proc Natl Acad Sci USA*, 98(12), 6917-6922.

Egan MF, Kojima M, Callicott JH, Goldberg TE, Kolachana BS, Bertolino A, Zaitsev E, Gold B, Goldman D, Dean M, Lu B, Weinberger DR. (2003). The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell*, 112(2), 257-269.

Elmenhorst D, Meyer PT, Matusch A, Winz OH, Zilles K, Bauer A. (2007). Test-retest stability of cerebral A(1) adenosine receptor quantification using [F-18]CPFPX and PET. *Eur J Nucl Med Mol Imaging*, 34(7), 1061-1070.

Ertel S (2005) The ball drawing test: Psi from untrodden ground. In: Parapsychology in the Twenty-First Century: Essays on the Future of Parapsychological Research (Thalbourne, M. A. S., L., ed), pp 90-123 Jefferson NC: McFarland.

Faraguna U, Vyazovskiy VV, Nelson AB, Tononi G, Cirelli C. (2008). A causal role for brain-derived neurotrophic factor in the homeostatic regulation of sleep. *J Neurosci*, 28(15), 4088-4095.

Faubel R, Lopez-Garcia E, Guallar-Castillon P, Graciani A, Banegas JR, Rodriguez-Artalejo F. (2009). Usual sleep duration and cognitive function in older adults in Spain. *J Sleep Res*, 18(4), 427-435.

Fellin T, Halassa MM, Terunuma M, Succol F, Takano H, Frank M, Moss SJ, Haydon PG. (2009). Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci U S A*, 106(35), 15037-15042.

Fifer WP, Byrd DL, Kaku M, Eigsti IM, Isler JR, Grose-Fifer J, Tarullo AR, Balsam PD. (2010). Newborn infants learn during sleep. *Proc Natl Acad Sci U S A*.

Finelli LA, Acherman P, Borbely AA. (2001a). Individual 'fingerprints' in human sleep EEG topography. *Neuropsychopharmacology*, 25, 57-62.

Finelli LA, Borbély AA, Achermann P. (2001b). Functional topography of the human nonREM sleep electroencephalogram. *Eur J Neurosci*, 13, 2282-2290.

Fischer MH, Mills RA, Shaki S. (2010). How to cook a SNARC: Number placement in text rapidly changes spatial-numerical associations. *Brain Cogn*, 72(3), 333-336.

Folstein MF, Folstein SE, McHugh PR. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*, 12(3), 189-198.

Fontinha BM, Diogenes MJ, Ribeiro JA, Sebastiao AM. (2008). Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. *Neuropharmacology*, 54(6), 924-933.

Fort P, Bassetti CL, Luppi PH. (2009). Alternating vigilance states: new insights regarding neuronal networks and mechanisms. *Eur J Neurosci*, 29(9), 1741-1753.

Foster PS, Drago V, Harrison DW. (2009). Assessment of nonverbal learning and memory using the Design Learning Test. *J Psychol*, 143(3), 245-266.

Franken P, Chollet D, Tafti M. (2001). The homeostatic regulation of sleep need is under genetic control. *J Neurosci*, 21(8), 2610-2621.

Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. (2000). Structure and function of adenosine receptors and their genes. *Naunyn Schmiedebergs Arch Pharmacol*, 362(4-5), 364-374.

Fredholm BB, Bättig K, Holmen J, Nehlig A, Zvartau EE. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacol Rev*, 51(1), 83-133.

Fredholm BB, Chen JF, Cunha RA, Svenningsson P, Vaugeois JM. (2005a). Adenosine and brain function. *Int Rev Neurobiol*, 63, 191-270.

Fredholm BB, Chen JF, Masino SA, Vaugeois JM. (2005b). Actions of adenosine at its receptors in the CNS: Insights from knockouts and drugs. *Annu Rev Pharmacol Toxicol*, 45, 385-412.

Fukunaga K, Miyamoto E. (2000). A working model of CaM kinase II activity in hippocampal long-term potentiation and memory. *Neurosci Res*, 38(1), 3-17.

Gallopin T, Luppi PH, Cauli B, Urade Y, Rossier J, Hayaishi O, Lambolez B, Fort P. (2005). The endogenous somnogen adenosine excites a subset of sleep-promoting neurons via A2A receptors in the ventrolateral preoptic nucleus. *Neuroscience*, 134(4), 1377-1390.

Gaus SE, Strecker RE, Tate BA, Parker RA, Saper CB. (2002). Ventrolateral preoptic nucleus contains sleep-active, galaninergic neurons in multiple mammalian species. *Neuroscience*, 115(1), 285-294.

Gazzaniga MS, Ivri RB, Mangun GR (2002) Cognitive Neuroscience: the biology of the mind. New York: W.W.Norton & Company, Inc.

Geiger A, Achermann P, Jenni OG. (2010). Association between sleep duration and intelligence scores in healthy children. *Dev Psychol*, 46(4), 949-954.

Gelman R, Gallistel CR (1978) The child's understanding of number: Cambridge Mass: Harward University Press.

Genoud C, Knott GW, Sakata K, Lu B, Welker E. (2004). Altered synapse formation in the adult somatosensory cortex of brain-derived neurotrophic factor heterozygote mice. *J Neurosci*, 24(10), 2394-2400.

Gobel SM, Calabria M, Farne A, Rossetti Y. (2006). Parietal rTMS distorts the mental number line: simulating 'spatial' neglect in healthy subjects. *Neuropsychologia*, 44(6), 860-868.

Goel N, Rao H, Durmer JS, Dinges DF. (2009). Neurocognitive consequences of sleep deprivation. *Semin Neurol*, 29(4), 320-339.

Golby AJ, Poldrack RA, Brewer JB, Spencer D, Desmond JE, Aron AP, Gabrieli JD. (2001). Material-specific lateralization in the medial temporal lobe and prefrontal cortex during memory encoding. *Brain*, 124, 1841-1854.

Gomez RL, Bootzin RR, Nadel L. (2006). Naps promote abstraction in language-learning infants. *Psychol Sci*, 17(8), 670-674.

Gottselig JM, Adam M, Rétey JV, Khatami R, Achermann P, Landolt H-P. (2006). Random number generation during sleep deprivation: Effects of caffeine on response maintenance and stereotypy. *J Sleep Res*, 15, 31-40.

Greene CM, Robertson IH, Gill M, Bellgrove MA. (2010). Dopaminergic genotype influences spatial bias in healthy adults. *Neuropsychologia*, 48(9), 2458-2464.

Greenwald AG, McGhee DE, Schwartz JL. (1998). Measuring individual differences in implicit cognition: the implicit association test. *J Pers Soc Psychol*, 74(6), 1464-1480.

Groeger JA, Zijlstra FR, Dijk DJ. (2004). Sleep quantity, sleep difficulties and their perceived consequences in a representative sample of some 2000 British adults. *J Sleep Res*, 13(4), 359-371.

Gruber R, Laviolette R, Deluca P, Monson E, Cornish K, Carrier J. (2010). Short sleep duration is associated with poor performance on IQ measures in healthy school-age children. *Sleep Med*, 11(3), 289-294.

Gunturkun O. (1997). Visual lateralization in birds: from neurotrophins to cognition? *Eur J Morphol*, 35(4), 290-302.

Hairston IS, Peyron C, Denning DP, Ruby NF, Flores J, Sapolsky RM, Heller HC, O'Hara BF. (2004). Sleep deprivation effects on growth factor expression in neonatal rats: a potential role for BDNF in the mediation of delta power. *J Neurophysiol*, 91(4), 1586-1595.

Halassa MM, Florian C, Fellin T, Munoz JR, Lee SY, Abel T, Haydon PG, Frank MG. (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron*, 61(2), 213-219.

Halassa MM, Haydon PG. (2010). Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*, 72, 335-355.

Hallanger AE, Levey AI, Lee HJ, Rye DB, Wainer BH. (1987). The origins of cholinergic and other subcortical afferents to the thalamus in the rat. *J Comp Neurol*, 262(1), 105-124.

Hanlon EC, Faraguna U, Vyazovskiy VV, Tononi G, Cirelli C. (2009). Effects of skilled training on sleep slow wave activity and cortical gene expression in the rat. *Sleep*, 32(6), 719-729.

Happe S, Klosch G, Lorenzo J, Kunz D, Penzel T, Roschke J, Himanen SL, Gruber G, Zeitlhofer J. (2005). Perception of sleep: subjective versus objective sleep parameters in patients with Parkinson's disease in comparison with healthy elderly controls. Sleep perception in Parkinson's disease and controls. *J Neurol*, 252(8), 936-943.

Hariri AR, Goldberg TE, Mattay VS, Kolachana BS, Callicott JH, Egan MF, Weinberger DR. (2003). Brain-derived neurotrophic factor val(66)met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci*, 23(17), 6690-6694.

Harrison Y, Horne JA. (1996). Sleep loss affects frontal lobe function, as shown in complex "real world" tasks. *Sleep Res*, 25, 467.

Harrison Y, Horne JA. (1997). Sleep deprivation affects speech. *Sleep*, 20, 871-877.

Harrison Y, Horne JA, Rothwell A. (2000). Prefrontal neuropsychological effects of sleep deprivation in young adults--a model for healthy aging? *Sleep*, 23(8), 1067-1073.

Hassainia F, Petit D, Nielsen T, Gauthier S, Montplaisir J. (1997). Quantitative EEG and statistical mapping of wakefulness and REM sleep in the evaluation of mild to moderate Alzheimer's disease. *Eur Neurol*, 37(4), 219-224.

Hättig H. (1992). Die Erfassung der spontanen initialen visuellen Explorationsasymmetrie [The assessment of a spontaneous initial visual exploration asymmetry; description and assessment]. *Zeitschrift für Neuropsychologie*, 1, 52-67.

Hayaishi O, Urade Y, Eguchi N, Huang Z-L. (2004). Genes for prostaglandin D synthase and receptor as well as adenosine A_{2A} receptor are involved in the homeostatic regulation of NREM sleep. *Arch Ital Biol*, 142, 533-539.

Haydon PG, Carmignoto G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev*, 86(3), 1009-1031.

He Y, Jones CR, Fujiki N, Xu Y, Guo B, Holder JL, Jr., Rossner MJ, Nishino S, Fu YH. (2009). The transcriptional repressor DEC2 regulates sleep length in mammals. *Science*, 325(5942), 866-870.

Hellige JB, Marks NL. (2001). Are hemispheric strategy differences independent of the level of performance? *Neuropsychology*, 15(3), 380-395.

Heuer H, Kohlisch O, Klein W. (2005). The effects of total sleep deprivation on the generation of random sequences of key-presses, numbers and nouns. *Q J Exp Psychol A*, 58(2), 275-307.
Hirschhorn R, Yang DR, Israni A. (1994). An Asp8Asn substitution results in the adenosine deaminase (ADA) genetic polymorphism (ADA 2 allozyme): occurrence on different chromosomal backgrounds and apparent intragenic crossover. *Ann Hum Genet*, 58(Pt 1), 1-9.
Hobson JA, McCarley RW, Wyzinski PW. (1975). Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science*, 189(4196), 55-58.
Hobson JA, Pace-Schott EF. (2002). The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nat Rev Neurosci*, 3(9), 679-693.
Horne JA. (1993). Human sleep, sleep loss and behaviour. Implications for the prefrontal cortex and psychiatric disorder. *Br. J. Psychiatry*, 162, 413-419.
Huang ZL, Qu WM, Eguchi N, Chen JF, Schwarzschild MA, Fredholm BB, Urade Y, Hayaishi O. (2005). Adenosine A_{2A}, but not A_1, receptors mediate the arousal effect of caffeine. *Nat Neurosci*, 8(7), 858-859.
Hubbard EM, Piazza M, Pinel P, Dehaene S. (2005). Interactions between number and space in parietal cortex. *Nat Rev Neurosci*, 6(6), 435-448.
Huber R, Ghilardi MF, Massimini M, Ferrarelli F, Riedner BA, Peterson MJ, Tononi G. (2006). Arm immobilization causes cortical plastic changes and locally decreases sleep slow wave activity. *Nat Neurosci*, 9(9), 1169-1176.
Huber R, Ghilardi MF, Massimini M, Tononi G. (2004). Local sleep and learning. *Nature*, 430(6995), 78-81.
Huber R, Tononi G, Cirelli C. (2007). Exploratory behavior, cortical BDNF expression, and sleep homeostasis. *Sleep*, 30(2), 129-139.
Illes P, Klotz KN, Lohse MJ. (2000). Signaling by extracellular nucleotides and nucleosides. *Naunyn Schmiedebergs Arch Pharmacol*, 362(4-5), 295-298.
Itagaki F, Niwa S, Itoh K, Momose T. (1995). Random number generation and the frontal cortex. *Int J Psychophysiol*, 19(1), 79-80.
Jahanshahi M, Dirnberger G, Fuller R, Frith CD. (2000). The role of the dorsolateral prefrontal cortex in random number generation: a study with positron emission tomography. *Neuroimage*, 12(6), 713-725.
Jenni OG, Caflisch JA, Molinari L (2009) A negative relationship between sleep duration and intellectual abilities in children and adolescents. In: Bianual Meeting of the Society of Research in Child Development Denver, CO.
Johns MW. (1991). A New Method for Measuring Daytime Sleepiness - the Epworth Sleepiness Scale. *Sleep*, 14(6), 540-545.
Jones BE (2005a) Basic mechanisms of sleep-wake states. In: Principles and Practise of Sleep Medicine (Kryger, M. H. et al., eds), pp 136-153 Philadelphia, PA: Elsevier Saunders.
Jones BE. (2005b). From waking to sleeping: neuronal and chemical substrates. *Trends Pharmacol Sci*, 26(11), 578-586.
Jones BE, Beaudet A. (1987). Distribution of acetylcholine and catecholamine neurons in the cat brainstem: a choline acetyltransferase and tyrosine hydroxylase immunohistochemical study. *J Comp Neurol*, 261(1), 15-32.
Jones K, Harrison Y. (2001). Frontal lobe function, sleep loss and fragmented sleep. *Sleep Med Rev*, 5(6), 463-475.
Kamei S, Morita A, Serizawa K, Mizutani T, Hirayanagi K. (2010). Quantitative EEG analysis of executive dysfunction in Parkinson disease. *J Clin Neurophysiol*, 27(3), 193-197.
Kandel ER, Schwartz JH, Jessel TM (eds.) (2000) Principles of neural science: McGraw-Hill.
Katzenberg D, Young T, Finn L, Lin L, King DP, Takahashi JS, Mignot E. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep*, 21, 569-576.
Kaufmann L, Koppelstaetter F, Siedentopf C, Haala I, Haberlandt E, Zimmerhackl LB, Felber S, Ischebeck A. (2006). Neural correlates of the number-size interference task in children. *Neuroreport*, 17(6), 587-591.
Kelley WM, Miezin FM, McDermott KB, Buckner RL, Raichle ME, Cohen NJ, Ollinger JM, Akbudak E, Conturo TE, Snyder AZ, Petersen SE. (1998). Hemispheric specialization in human dorsal frontal cortex and medial temporal lobe for verbal and nonverbal memory encoding. *Neuron*, 20(5), 927-936.
Kirchner WK. (1958). Age differences in short-term retention of rapidly changing information. *J Exp Psychol*, 55(4), 352-358.
Klimesch W. (1996). Memory Processes, Brain Oscillations and EEG Synchronization [Review]. *Int J Psychophysiol*, 24(1-2), 61-100.
Klimesch W. (1999). EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Rev*, 29(2-3), 169-195.

Kline CE, Zielinski MR, Devlin TM, Kripke DF, Bogan RK, Youngstedt SD. (2010). Self-reported long sleep in older adults is closely realted to objective time in bed. *Sleep and Biological Rhythms*, 8, 42-51.

Knops A, Thirion B, Hubbard EM, Michel V, Dehaene S. (2009). Recruitment of an area involved in eye movements during mental arithmetic. *Science*, 324(5934), 1583-1585.

Korte M, Carroll P, Wolf E, Brem G, Thoenen H, Bonhoeffer T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A*, 92(19), 8856-8860.

Koten JW, Jr., Wood G, Hagoort P, Goebel R, Propping P, Willmes K, Boomsma DI. (2009). Genetic contribution to variation in cognitive function: an FMRI study in twins. *Science*, 323(5922), 1737-1740.

Kreiner WA. (2003). On the Newcomb-Benford Law. *Zeitschrift für Naturforschung Section A-A Journal of Physical Sciences*, 58(11), 618-622.

Kronholm E, Sallinen M, Suutama T, Sulkava R, Era P, Partonen T. (2009). Self-reported sleep duration and cognitive functioning in the general population. *J Sleep Res*, 18(4), 436-446.

Krueger JM, Rector DM, Roy S, Van Dongen HP, Belenky G, Panksepp J. (2008). Sleep as a fundamental property of neuronal assemblies. *Nat Rev Neurosci*, 9(12), 910-919.

Landolt HP. (2008a). Genotype-dependent differences in sleep, vigilance, and response to stimulants. *Curr Pharm Des*, 14(32), 3396-3407.

Landolt HP. (2008b). Sleep homeostasis: A role for adenosine in humans? *Biochem Pharmacol*, 75(11), 2070-2079.

Landolt HP, Dijk DJ, Gaus SE, Borbély AA. (1995). Caffeine reduces low-frequency delta activity in the human sleep EEG. *Neuropsychopharmacology*, 12(3), 229-238.

Landolt HP, Rétey JV, Tönz K, Gottselig JM, Khatami R, Buckelmüller I, Achermann P. (2004). Caffeine attenuates waking and sleep electroencephalographic markers of sleep homeostasis in humans. *Neuropsychopharmacology*, 29, 1933-1939.

Landsness EC, Crupi D, Hulse BK, Peterson MJ, Huber R, Ansari H, Coen M, Cirelli C, Benca RM, Ghilardi MF, Tononi G. (2009). Sleep-dependent improvement in visuomotor learning: a causal role for slow waves. *Sleep*, 32(10), 1273-1284.

Latini S, Pedata F. (2001). Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem*, 79(3), 463-484.

Lauderdale DS, Knutson KL, Yan LL, Liu K, Rathouz PJ. (2008). Self-reported and measured sleep duration: how similar are they? *Epidemiology*, 19(6), 838-845.

Lezak M (1983) Neuropsychological assessment. (Press., N. Y. O. U., ed).

Lim JL, Dinges DF. (2008). Sleep deprivation and vigilant attention. *Ann N Y Acad Sci*, 1129, 305-322.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E. (1999). The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell*, 98(3), 365-376.

Lindskog M, Svenningsson P, Pozzi L, Kim Y, Fienberg AA, Bibb JA, Fredholm BB, Nairn AC, Greengard P, Fisone G. (2002). Involvement of DARPP-32 phosphorylation in the stimulant action of caffeine.[see comment]. *Nature*, 418(6899), 774-778.

Loetscher T, Bockisch CJ, Nicholls ME, Brugger P. (2010). Eye position predicts what number you have in mind. *Curr Biol*, 20(6), R264-265.

Loetscher T, Brugger P. (2007). Exploring number space by random digit generation. *Exp Brain Res*, 180(4), 655-665.

Loetscher T, Brugger P. (2009). Random number generation in neglect patients reveals enhanced response stereotypy, but no neglect in number space. *Neuropsychologia*, 47(1), 276-279.

Loetscher T, Schwarz U, Schubiger M, Brugger P. (2008). Head turns bias the brain's internal random generator. *Curr Biol*, 18(2), R60-62.

Longo MR, Lourenco SF. (2007). Spatial attention and the mental number line: evidence for characteristic biases and compression. *Neuropsychologia*, 45(7), 1400-1407.

Lu B. (2003). Pro-region of neurotrophins: role in synaptic modulation. *Neuron*, 39(5), 735-738.

Lu Y, Christian K, Lu B. (2008). BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiol Learn Mem*, 89(3), 312-323.

Luciano M, Zhu G, Kirk KM, Gordon SD, Heath AC, Montgomery GW, Martin NG. (2007). "No thanks, it keeps me awake": The genetics of coffee-attributed sleep disturbance. *Sleep*, 30(10), 1378-1386.

Luikart BW, Parada. (2006). Receptor tyrosine kinase B-mediated excitatory synaptogenesis *Prog Brain Res*, 157, 15-24.

Luppi PH. (2010). Neurochemical aspects of sleep regulation with specific focus on slow-wave sleep. *World J Biol Psychiatry*, 11 Suppl 1, 4-8.

Mackiewicz M, Nikonova EV, Zimmerman JE, Galante RJ, Zhang L, Cater JR, Geiger JD, Pack AI. (2003). Enzymes of adenosine metabolism in the brain: diurnal rhythm and the effect of sleep deprivation. *J Neurochem*, 85(2), 348-357.

Maquet P. (2001). The role of sleep in learning and memory. *Science*, 294(5544), 1048-1052.

Maret S, Dorsaz S, Gurcel L, Pradervand S, Petit B, Pfister C, Hagenbuchle O, O'Hara BF, Franken P, Tafti M. (2007). Homer1a is a core brain molecular correlate of sleep loss. *Proc Natl Acad Sci U S A*, 104(50), 20090-20095.

Marks GA, Birabil CG. (1998). Enhancement of rapid eye movement sleep in the rat by cholinergic and adenosinergic agonists infused into the pontine reticular formation. *Neuroscience*, 86(1), 29-37.

McCarley RW, Massaquoi SG. (1992). Neurobiological structure of the revised limit cycle reciprocal interaction model of REM cycle control. *J Sleep Res*, 1, 132-137.

McCarthy ME, Waters WF. (1997). Decreased attentional responsivity during sleep deprivation: orienting response latency, amplitude, and habituation. *Sleep*, 20(2), 115-123.

McCormick DA, Pape HC. (1990). Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurons. *J Physiol*, 431, 291-318.

McGinty D, Gong H, Suntsova N, Alam MN, Methippara M, Guzman-Marin R, Szymusiak R. (2004). Sleep-promoting functions of the hypothalamic median preoptic nucleus: inhibition of arousal systems. *Arch Ital Biol*, 142(4), 501-509.

McGinty DJ, Harper RM. (1976). Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res*, 101(3), 569-575.

McNair DM, Lorr M, Doppleman LF (1971) Edits Manual for the Profile of Mood States (POMS): Educational and Industrial Testing Service, San Diego, CA.

Mignot E, Lammers GJ, Ripley B, Okun M, Nevsimalova S, Overeem S, Vankova J, Black J, Harsh J, Bassetti C, Schrader H, Nishino S. (2002). The role of cerebrospinal fluid hypocretin measurement in the diagnosis of narcolepsy and other hypersomnias. *Arch Neurol*, 59(10), 1553-1562.

Miller GA. (1956). The magical number seven plus or minus two: some limits on our capacity for processing information. *Psychol Rev*, 63(2), 81-97.

Minichiello L. (2009). TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci*, 10(12), 850-860.

Mishima K, Tozawa T, Satoh K, Saitoh H, Mishima Y. (2005). The 3111T/C polymorphism of hClock is associated with evening preference and delayed sleep timing in a Japanese population sample. *Am J Med Genet B Neuropsychiatr Genet*, 133B(1), 101-104.

Mochizuki T, Crocker A, McCormack S, Yanagisawa M, Sakurai T, Scammell TE. (2004). Behavioral state instability in orexin knock-out mice. *J Neurosci*, 24(28), 6291-6300.

Mograss MA, Guillem F, Stickgold R. (2010). Individual differences in face recognition memory: comparison among habitual short, average, and long sleepers. *Behav Brain Res*, 208(2), 576-583.

Montag C, Weber B, Jentgens E, Elger C, Reuter M. (2010). An epistasis effect of functional variants on the BDNF and DRD2 genes modulates gray matter volume of the anterior cingulate cortex in healthy humans. *Neuropsychologia*, 48(4), 1016-1021.

Monti JM. (1982). Catecholamines and the sleep-wake cycle. I. EEG and behavioral arousal. *Life Sci.*, 30, 1145-1157.

Moore RY, Eichler VB. (1972). Loss of a circadian adrenal corticosterone rhythm following suprachiasmatic lesions in the rat. *Brain Res*, 42, 201-206.

Moruzzi G, Magoun HW. (1949). Brain stem reticular formation and activation of the EEG. *Electroencephalogr Clin Neurophysiol*, 1(4), 455-473.

Nagappan G, Lu B. (2005). Activity-dependent modulation of the BDNF receptor TrkB: mechanisms and implications. *Trends Neurosci*, 28(9), 464-471.

Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology*, 32(4), 392-401.

Nemoto K, Ohnishi T, Mori T, Moriguchi Y, Hashimoto R, Asada T, Kunugi H. (2006). The Val66Met polymorphism of the brain-derived neurotrophic factor gene affects age-related brain morphology. *Neurosci Lett*, 397(1-2), 25-29.

Newcomb S. (1881). Astronomical Observatories. *Science*, 2(60), 377-380.

Nieder A. (2009). Prefrontal cortex and the evolution of symbolic reference. *Curr Opin Neurobiol*, 19(1), 99-108.

Nieder A, Dehaene S. (2009). Representation of number in the brain. *Annu Rev Neurosci*, 32, 185-208.

Ninan I, Bath KG, Dagar K, Perez-Castro R, Plummer MR, Lee FS, Chao MV. (2010). The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *J Neurosci*, 30(26), 8866-8870.

Nishino S, Ripley B, Overeem S, Lammers GJ, Mignot E. (2000). Hypocretin (orexin) deficiency in human narcolepsy. *Lancet*, 355(9197), 39-40.
Nixon GM, Thompson JM, Han DY, Becroft DM, Clark PM, Robinson E, Waldie KE, Wild CJ, Black PN, Mitchell EA. (2008). Short sleep duration in middle childhood: risk factors and consequences. *Sleep*, 31(1), 71-78.
Oakley N (1997) Validation with Polysomnography of the Sleepwatch Sleep/Wake Scoring Algorithm used by the Actiwatch Activity Monitoring System: Cambridge Neurotechnology Ltd, Upper Pendrill Court, Ermine St North, Papweorth Everard, Cambridge CB3 8UY, UK.
Ohayon MM, Carskadon MA, Guilleminault C, Vitiello MV. (2004). Meta-analysis of quantitative sleep parameters from childhood to old age in healthy individuals: developing normative sleep values across the human lifespan. *Sleep*, 27(7), 1255-1273.
Okada T, Mochizuki T, Huang ZL, Eguchi N, Sugita Y, Urade Y, Hayaishi O. (2003). Dominant localization of adenosine deaminase in leptomeninges and involvement of the enzyme in sleep. *Biochem Biophys Res Commun*, 312(1), 29-34.
Oken BS, Salinsky MC, Elsas SM. (2006). Vigilance, alertness, or sustained attention: physiological basis and measurement. *Clin Neurophysiol*, 117(9), 1885-1901.
Oliet SH, Mothet JP. (2006). Molecular determinants of D-serine-mediated gliotransmission: from release to function. *Glia*, 54(7), 726-737.
Oliveri M, Rausei V, Koch G, Torriero S, Turriziani P, Caltagirone C. (2004). Overestimation of numerical distances in the left side of space. *Neurology*, 63(11), 2139-2141.
Pace-Schott EF, Hobson JA. (2002). The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nat Rev Neurosci*, 3(8), 591-605.
Palchykova S, Winsky-Sommerer R, Shen HY, Boison D, Gerling A, Tobler I. (2010). Manipulation of adenosine kinase affects sleep regulation in mice. *J Neurosci*, 30(39), 13157-13165.
Perret E. (1974). The left frontal lobe of man and the suppression of habitual responses in verbal categorical behaviour. *Neuropsychologia*, 12(3), 323-330.
Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo MT, Pascucci T, Puglisi-Allegra S, Reichelt KL, Conciatori M, Baldi A, Keller F. (2000). Adenosine deaminase alleles and autistic disorder: case-control and family-based association studies. *Am J Med Genet*, 96(6), 784-790.
Peyron C, Wurts SW, Srere HK, Heller HC, Edgar DM, Kilduff TS. (1998). mRNA level of brain-derived neurotrophic factor increases in several brain regions after sleep deprivation. *Soc Neurosci Abstr*, 24, 1430.
Pinhas M, Fischer MH. (2008). Mental movements without magnitude? A study of spatial biases in symbolic arithmetic. *Cognition*, 109(3), 408-415.
Porkka-Heiskanen T, Strecker RE, McCarley RW. (2000). Brain site-specificity of extracellular adenosine concentration changes during sleep deprivation and spontaneous sleep: an in vivo microdialysis study. *Neuroscience*, 99(3), 507-517.
Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW. (1997). Adenosine: a mediator of the sleep-inducing effects of prolonged wakefulness. *Science*, 276, 1265-1268.
Powell HW, Koepp MJ, Symms MR, Boulby PA, Salek-Haddadi A, Thompson PJ, Duncan JS, Richardson MP. (2005). Material-specific lateralization of memory encoding in the medial temporal lobe: blocked versus event-related design. *Neuroimage*, 27(1), 231-239.
Priftis K, Zorzi M, Meneghello F, Marenzi R, Umilta C. (2006). Explicit versus implicit processing of representational space in neglect: dissociations in accessing the mental number line. *J Cogn Neurosci*, 18(4), 680-688.
Radulovacki M, Virus RM, Djuricic-Nedelson M, Green RD. (1983). Hypnotic Effects of Deoxycorformycin in Rats. *Brain Research*, 271(2), 392-395.
Ravnkilde B, Videbech P, Rosenberg R, Gjedde A, Gade A. (2002). Putative tests of frontal lobe function: a PET-study of brain activation during Stroop's Test and verbal fluency. *J Clin Exp Neuropsychol*, 24(4), 534-547.
Rechtschaffen A, Kales A (1968) A manual of standardized terminology, techniques and scoring system for sleep stages of human subjects. Bethesda, Maryland: National Institutes of Health.
Regard M, Strauss E, Knapp P. (1982). Children's production on verbal and non-verbal fluency tasks. *Percept Mot Skills*, 55(3), 839-844.
Regel SJ, Gottselig JM, Schuderer J, Tinguely G, Retey JV, Kuster N, Landolt HP, Achermann P. (2007). Pulsed radio frequency radiation affects cognitive performance and the waking electroencephalogram. *Neuroreport*, 18(8), 803-807.

Rétey JV, Adam M, Honegger E, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P. (2005). A functional genetic variation of adenosine deaminase affects the duration and intensity of deep sleep in humans. *Proc Natl Acad Sci U S A*, 102(43), 15676-15681.

Rétey JV, Adam M, Khatami R, Luhmann UFO, Jung HH, Berger W, Landolt H-P. (2007). A Genetic Variation in the Adenosine A_{2A} Receptor Gene (*ADORA2A*) Contributes to Individual Sensitivity to Caffeine Effects on Sleep. *Clin Pharmacol Ther*, 81, 692-698.

Riksen NP, Franke B, van den Broek P, Naber M, Smits P, Rongen GA. (2008). The 22G>A polymorphism in the adenosine deaminase gene impairs catalytic function but does not affect reactive hyperaemia in humans in vivo. *Pharmacogenet Genomics*, 18(10), 843-846.

Roenneberg T, Wirz-Justice A, Merrow M. (2003). Life between clocks: Daily temporal patterns of human chronotypes. *J Biol Rhythms*, 18(1), 80-90.

Roffwarg HP, Muzio JN, Dement WC. (1966). Ontogenetic development of the human sleep-dream cycle. *Science*, 152, 604-619.

Rogers L, Andrew R (2002) Comparative vertebrate lateralization. Cambridge, UK.

Ruff RM, Allen CC, Farrow CE, Niemann H, Wylie T. (1994). Figural fluency: differential impairment in patients with left versus right frontal lobe lesions. *Archives of Clinical Neuropsychology*, 9(1), 41-55.

Rusterholz T, Durr R, Achermann P. (2010). Inter-individual differences in the dynamics of sleep homeostasis. *Sleep*, 33(4), 491-498.

Sadeh A, Acebo C. (2002). The role of actigraphy in sleep medicine. *Sleep Med Rev*, 6(2), 113-124.

Sadeh A, Alster J, Urbach D, Lavie P. (1989). Actigraphically based automatic bedtime sleep-wake scoring: Validity and clinical applications. *J Ambulatory Monitoring*, 2, 209-216.

Sadeh A, Hauri PJ, Kripke DF, Lavie P. (1995). The role of actigraphy in the evaluation of sleep disorders. *Sleep*, 18(4), 288-302.

Sagaspe P, Charles A, Taillard J, Bioulac B, Philip P. (2003). [Inhibition and working memory: effect of acute sleep deprivation on a random letter generation task]. *Can J Exp Psychol*, 57(4), 265-273.

Sakurai T. (2007). The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci*, 8(3), 171-181.

Sanger F, Nicklen S, Coulson AR. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A*, 74(12), 5463-5467.

Saper CB, Chou TC, Scammell TE. (2001). The sleep switch: hypothalamic control of sleep and wakefulness. *Trends Neurosci*, 24(12), 726-731.

Saper CB, Scammell TE, Lu J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 437(7063), 1257-1263.

Savitz J, Solms M, Ramesar R. (2006). The molecular genetics of cognition: dopamine, COMT and BDNF. *Genes Brain Behav*, 5(4), 311-328.

Schabus M, Hodlmoser K, Gruber G, Sauter C, Anderer P, Klosch G, Parapatics S, Saletu B, Klimesch W, Zeitlhofer J. (2006). Sleep spindle-related activity in the human EEG and its relation to general cognitive and learning abilities. *Eur J Neurosci* 23(7), 1738-1746.

Scharf MT, Naidoo N, Zimmerman JE, Pack AI. (2008). The energy hypothesis of sleep revisited. *Prog Neurobiol*, 86(3), 264-280.

Schmidt C, Collette F, Cajochen C, Peigneux P. (2007). A time to think: Circadian rhythms in human cognition. *Cogn Neuropsychol*, 24(7), 755-789.

Schwierin B, Borbély AA, Tobler I. (1996). Effects of N^6-cyclopentyladenosine and caffeine on sleep regulation in the rat. *Eur J Pharmacol*, 300(3), 163-171.

Sebastiao AM, Ribeiro JA. (2009). Triggering neurotrophic factor actions through adenosine A2A receptor activation: implications for neuroprotection. *Br J Pharmacol*, 158(1), 15-22.

Seugnet L, Boero J, Gottschalk L, Duntley SP, Shaw PJ. (2006). Identification of a biomarker for sleep drive in flies and humans. *Proc Natl Acad Sci U S A*, 103(52), 19913-19918.

Sherin JE, Shiromani PJ, McCarley RW, Saper CB. (1996). Activation of ventrolateral preoptic neurons during sleep. *Science*, 271, 216-219.

Siegel J. (2004a). Brain mechanisms that control sleep and waking. *Naturwiss.*, 91(8), 355-365.

Siegel JM. (2004b). The neurotransmitters of sleep. *J Clin Psychiatry*, 65 Suppl 16, 4-7.

Silva GE, Goodwin JL, Sherrill DL, Arnold JL, Bootzin RR, Smith T, Walsleben JA, Baldwin CM, Quan SF. (2007). Relationship between reported and measured sleep times: the sleep heart health study (SHHS). *J Clin Sleep Med*, 3(6), 622-630.

Sinton CM, McCarley RW. (2004). Neurophysiological mechanisms of sleep and wakefulness: a question of balance. *Semin Neurol*, 24(3), 211-223.

Sivertsen B, Omvik S, Havik OE, Pallesen S, Bjorvatn B, Nielsen GH, Straume S, Nordhus IH. (2006). A comparison of actigraphy and polysomnography in older adults treated for chronic primary insomnia. *Sleep*, 29(10), 1353-1358.

Somers VK, Dyken ME, Mark AL, Abboud FM. (1993). Sympathetic-nerve activity during sleep in normal subjects. *N Engl J Med.*, 328, 303-307.

Spielberger CD, Gorsuch RL, Lushene RE (1970) Manual for the State-Trait Anxiety Inventory. Palo Alto, Calif.: Consulting Psychologists Press.

Stephan FK, Zucker I. (1972). Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. *Proc Natl Acad Sci U S A*, 69, 1583-1586.

Steriade M. (2003). The corticothalamic system in sleep. *Front Biosci*, 8, 878-899.

Steriade M, Curró Dossi R, Nuñez A. (1991). Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: Cortically induced synchronization and brainstem cholinergic suppression. *J Neurosci*, 11, 3200-3217.

Steriade M, Jones EG, Llinás RR (1990) Thalamic oscillations and signaling. New York: Wiley.

Steriade M, McCormick DA, Sejnowski TJ. (1993a). Thalamocortical oscillations in the sleeping and aroused brain. *Science*, 262, 679-685.

Steriade M, Nuñez A, Amzica F. (1993b). Intracellular analysis of relations between the slow (<1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. *J Neurosci*, 13, 3266-3283.

Steriade M, Nuñez A, Amzica F. (1993c). A novel slow (<1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. *J Neurosci*, 13, 3252-3265.

Strauss E, Shermann E, Spreen O (2006) A Compendium of Neuropsychological Tests: Oxford University Press, Inc.

Stroop J. (1935). Studies of interference in serial verbal reactions. *J Exp Psychol*, 18(643).

Sturm A, Clarenbach P (1997) Schlafstörungen. Stuttgart, New York: Thieme Verlag.

Sun T, Patoine C, Abu-Khalil A, Visvader J, Sum E, Cherry TJ, Orkin SH, Geschwind DH, Walsh CA. (2005). Early asymmetry of gene transcription in embryonic human left and right cerebral cortex. *Science*, 308(5729), 1794-1798.

Tabor HK, Risch NJ, Myers RM. (2002). Candidate-gene approaches for studying complex genetic traits: practical considerations. *Nat Rev Genet*, 3(5), 391-397.

Tafti M. (2009). Genetic aspects of normal and disturbed sleep. *Sleep Med*, 10 Suppl 1, S17-21.

Tang Y, Chorlian DB, Rangaswamy M, O'Connor S, Taylor R, Rohrbaugh J, Porjesz B, Begleiter H. (2007). Heritability of bipolar EEG spectra in a large sib-pair population. *Behav Genet*, 37(2), 302-313.

Tebano MT, Martire A, Rebola N, Pepponi R, Domenici MR, Gro MC, Schwarzschild MA, Chen JF, Cunha RA, Popoli P. (2005). Adenosine A2A receptors and metabotropic glutamate 5 receptors are co-localized and functionally interact in the hippocampus: a possible key mechanism in the modulation of N-methyl-D-aspartate effects. *J Neurochem*, 95(4), 1188-1200.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM. (2000). Reduced number of hypocretin neurons in human narcolepsy. *Neuron*, 27(3), 469-474.

Thomas M, Sing H, Belenky G, Holcomb H, Mayberg H, Dannals R, Wagner H, Thorne D, Popp K, Rowland L, Welsh A, Balwinski S, Redmond D. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *J Sleep Res*, 9(4), 335-352.

Tinguely G, Finelli LA, Landolt HP, Borbely AA, Achermann P. (2006). Functional EEG topography in sleep and waking: state-dependent and state-independent features. *Neuroimage*, 32(1), 283-292.

Tischfield JA, Creagan RP, Nichols EA, Ruddle FH. (1974). Assignment of a gene for adenosine deaminase to human chromosome 20. *Hum Hered*, 24(1), 1-11.

Tononi G, Cirelli C. (2003). Sleep and synaptic homeostasis: a hypothesis. *Brain Res Bull*, 62(2), 143-150.

Tononi G, Cirelli C. (2006). Sleep function and synaptic homeostasis. *Sleep Med Rev*, 10(1), 49-62.

Torsvall L, Åkerstedt T. (1987). Sleepiness on the job: Continuously measured EEG changes in train drivers. *Electroenceph clin Neurophysiol*, 66, 502-511.

Towse JN. (1998). On random generation and the central executive of working memory. *Br J Psychol*, 89 (Pt 1), 77-101.

Towse JN, Neil D. (1998). Analyzing human random generation behavior: A review of methods used and a computer program for describing performance. *Behavior Research Methods Instruments, & Computers*, 30(4), 583-591.

Tucker AM, Dinges DF, Van Dongen HPA. (2007). Trait interindividual differences in the sleep physiology of healthy young adults. *J Sleep Res*, 16, 170-180.

Tunbridge EM, Harrison PJ, Weinberger DR. (2006). Catechol-o-methyltransferase, cognition, and psychosis: Val(158)Met and beyond. *Biol Psychiatry*, 60(2), 141-151.
Turrigiano GG, Nelson SB. (2000). Hebb and homeostasis in neuronal plasticity. *Curr Opin Neurobiol*, 10(3), 358-364.
Tyler WJ, Alonso M, Bramham CR, Pozzo-Miller LD. (2002). From acquisition to consolidation: on the role of brain-derived neurotrophic factor signaling in hippocampal-dependent learning. *Learn Mem*, 9(5), 224-237.
Urade Y, Eguchi N, Qu W-M, Sakata M, Huang ZL, Chen JF, Schwarzschild MA, Fink JS, Hayaishi O. (2003). Sleep regulation in adenosine A_{2A} receptor-deficient mice. *Neurology*, 61, S94-S96.
Uttl B, Pilkenton-Taylor C. (2001). Letter cancellation performance across the adult life span. *Clin Neuropsychol*, 15(4), 521-530.
van Beijsterveldt CE, Molenaar PC, de Geus EJ, Boomsma DI. (1996). Heritability of human brain functioning as assessed by electroencephalography. *Am J Hum Genet*, 58(3), 562-573.
Van Den Berg JF, Van Rooij FJ, Vos H, Tulen JH, Hofman A, Miedema HM, Neven AK, Tiemeier H. (2008). Disagreement between subjective and actigraphic measures of sleep duration in a population-based study of elderly persons. *J Sleep Res*, 17(3), 295-302.
Van Der Werf YD, Altena E, Schoonheim MM, Sanz-Arigita EJ, Vis JC, De Rijke W, Van Someren EJ. (2009). Sleep benefits subsequent hippocampal functioning. *Nat Neurosci*, 12(2), 122-123.
van Dijck JP, Gevers W, Fias W. (2009). Numbers are associated with different types of spatial information depending on the task. *Cognition*, 113(2), 248-253.
Van Dongen HP, Dinges DF. (2003). Investigating the interaction between the homeostatic and circadian processes of sleep-wake regulation for the prediction of waking neurobehavioural performance. *J Sleep Res*, 12(3), 181-187.
Van Dongen HP, Maislin G, Mullington JM, Dinges DF. (2003). The cumulative cost of additional wakefulness: dose-response effects on neurobehavioral functions and sleep physiology from chronic sleep restriction and total sleep deprivation. *Sleep*, 26(2), 117-126.
Van Dongen HP, Vitellaro KM, Dinges DF. (2005). Individual differences in adult human sleep and wakefulness: Leitmotiv for a research agenda. *Sleep*, 28(4), 479-496.
Van Dongen HPA, Baynard MD, Maislin G, Dinges DF. (2004). Systematic interindividual differences in neurobehavioral impairment from sleep loss: Evidence of trait-like differential vulnerability. *Sleep*, 27(3), 423-433.
van Stegeren A, Rohleder N, Everaerd W, Wolf OT. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: effect of betablockade. *Psychoneuroendocrinology*, 31(1), 137-141.
Ventriglia M, Bocchio Chiavetto L, Benussi L, Binetti G, Zanetti O, Riva MA, Gennarelli M. (2002). Association between the BDNF 196 A/G polymorphism and sporadic Alzheimer's disease. *Mol Psychiatry*, 7(2), 136-137.
Viola AU, Archer SN, James LM, Groeger JA, Lo JC, Skene DJ, von Schantz M, Dijk DJ. (2007). PER3 polymorphism predicts sleep structure and waking performance. *Current Biology*, 17(7), 613-618.
Von Economo C. (1930). Sleep as a problem of localization. *J Nerv Ment Dis*, 71, 249-259.
von Zerssen D, Doerr P, Emrich HM, Lund R, Pirke KM. (1987). Diurnal variation of mood and the cortisol rhythm in depression and normal states of mind. *Eur Arch Psychiatr Neurol Sci*, 237, 36-45.
Walker MP, Stickgold R. (2006). Sleep, memory, and plasticity. *Annu Rev Psychol*, 57, 139-166.
Waterhouse EG, Xu B. (2009). New insights into the role of brain-derived neurotrophic factor in synaptic plasticity. *Mol Cell Neurosci*, 42(2), 81-89.
Watson NF, Kapur V, Arguelles LM, Goldberg J, Schmidt DF, Armitage R, Buchwald D. (2003). Comparison of subjective and objective measures of insomnia in monozygotic twins discordant for chronic fatigue syndrome. *Sleep*, 26(3), 324-328.
Webb WB. (1979). Are short and long sleepers different? *Psychol Rep*, 44, 259-264.
Weintraub S, Mesulam MM. (1988). Visual hemispatial inattention: stimulus parameters and exploratory strategies. *J Neurol Neurosurg Psychiatry*, 51(12), 1481-1488.
Wisor JP, Eriksson KS. (2005). Dopaminergic-adrenergic interactions in the wake promoting mechanism of modafinil. *Neuroscience*, 132(4), 1027-1034.
Wood G, Fischer MH. (2008). Numbers, space, and action - from finger counting to the mental number line and beyond. *Cortex*, 44(4), 353-358.
Wood G, Nuerk HC, Willmes K. (2006). Crossed hands and the SNARC effect: a failure to replicate Dehaene, Bossini and Giraux (1993). *Cortex*, 42(8), 1069-1079; discussion 1119-1023.

Wood G, Nuerk HC, Willmes K, Fischer MH. (2008). On the cognitive link between space and number: A meta-analysis of the SNARC effect. *Psychol Sci Q*, 50(4), 489-525.

Yaar R, Jones MR, Chen JF, Ravid K. (2005). Animal models for the study of adenosine receptor function. *J Cell Physiol*, 202(1), 9-20.

Yamada K, Nabeshima T. (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J Pharmacol Sci*, 91(4), 267-270.

Yovel G, Tambini A, Brandman T. (2008). The asymmetry of the fusiform face area is a stable individual characteristic that underlies the left-visual-field superiority for faces. *Neuropsychologia*, 46(13), 3061-3068.

Zorzi M, Priftis K, Meneghello F, Marenzi R, Umilta C. (2006). The spatial representation of numerical and non-numerical sequences: evidence from neglect. *Neuropsychologia*, 44(7), 1061-1067.

Zorzi M, Priftis K, Umilta C. (2002). Brain damage: neglect disrupts the mental number line. *Nature*, 417(6885), 138-139.

Zuccato C, Cattaneo E. (2009). Brain-derived neurotrophic factor in neurodegenerative diseases. *Nat Rev Neurol*, 5(6), 311-322.